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THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

nature

nature 452, 253-386 20 March 2008

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HOT JUPITERS
Organic molecules identified

HUMAN COOPERATION
Victors don't punish

SPRING FEVER
The hormone that sparks the need to breed

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The struggle to match supply and demand

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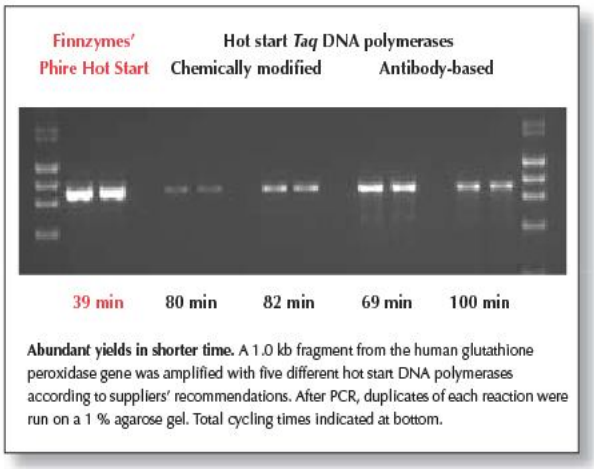

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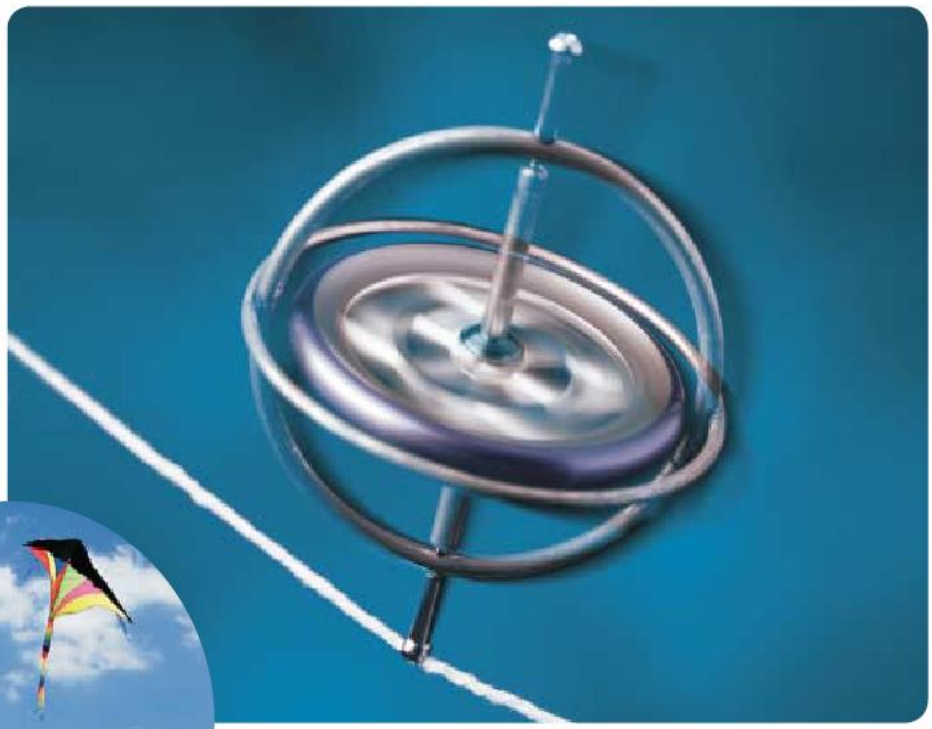

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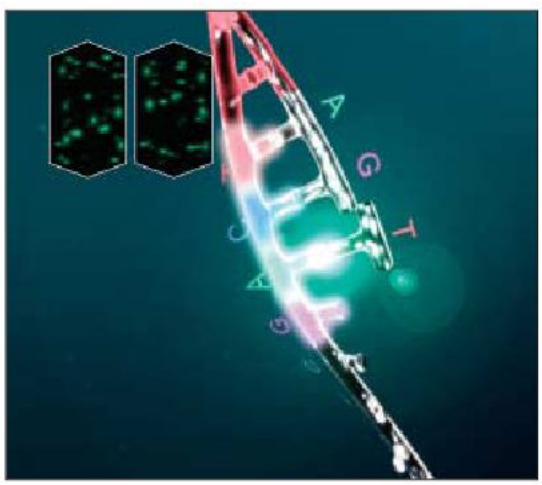

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In the US Nature (ISSN 0028-0836) is published weekly on Thursday, except the last week in December by Nature Publishing Group, 75 Varick St, 9th Fl, New York NY 10013-1917, USA. US Periodicals postage paid at New York NY, and additional mailing post offices. US POSTMASTER: send address changes to Nature, Subscription Dept, 342 Broadway PMB 301, New York NY 10013-3910, USA.
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Worth its salt: desalination to the rescue? News, p. **260**.

NASA JOHNSON SPACE CENTER

Picking up the pieces: Genesis data retrieved. News, p. **259**.

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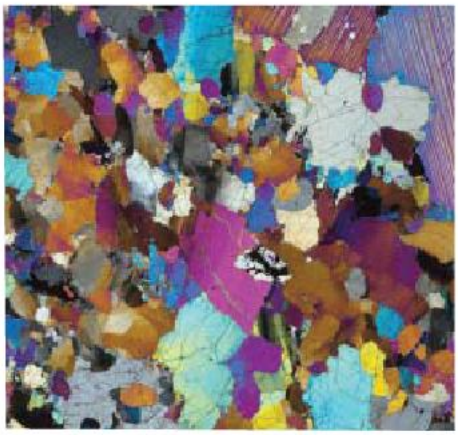

Rock of ages: freshly dredged samples throw light onto mantle recycling, p. **311**.

NATURE ONLINE

ADVANCE ONLINE PUBLICATION

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NAD synthase NMNAT acts as a chaperone to protect against neurodegeneration
R G Zhai *et al.* doi:10.1038/nature06721

Variations in DNA elucidate molecular networks that cause disease
Y Chen *et al.* doi:10.1038/nature06757

Genetics of gene expression and its effect on disease
V Emilsson *et al.* doi:10.1038/nature06758

Tumour maintenance is mediated by eNOS
K-H Lim, B B Ancrile, D F Kashatus & C M Counter
doi:10.1038/nature06778

A neural representation of depth from motion parallax in macaque visual cortex
J W Nadler, D E Angelaki & G C DeAngelis doi:10.1038/nature06814

Lateral presynaptic inhibition mediates gain control in an olfactory circuit
S R Olsen & R I Wilson doi:10.1038/nature06864

PUBLISHED ON 19 MARCH 2008

Chromatin dynamics during epigenetic reprogramming in the mouse germ line
P Hajkova *et al.* doi:10.1038/nature06714

Integration of growth and specification in chick wing digit-patterning
M Towers, R Mahood, Y Yin & C Tickle doi:10.1038/nature06718

Hydatellaceae are water lilies with gymnospermous tendencies
W E Friedman doi:10.1038/nature06733

Kemp elimination catalysts by computational enzyme design
D Röthlisberger *et al.* doi:10.1038/nature06879

BRIEF COMMUNICATIONS ARISING

PUBLISHED ON 20 MARCH 2008

Arising from 'Damage to the prefrontal cortex increases utilitarian moral judgements' by M Koenigs *et al. Nature* **446,** 908–911 (2007).

Do abnormal responses show utilitarian bias?
G Kahane & N Shackel doi:10.1038/nature06785

Reply: Koenigs *et al.* doi:10.1038/nature06804

WATER UNDER PRESSURE

The special content in this week's print and online issues on the global water supply crisis is backed-up by comment pieces on the podcast. Also this week, the discovery of organic molecules on an exoplanet, and the evolution of magnanimous behaviour in 'winners'. Subscribe —it's free — to the weekly programme or download single copies from iTunes or from:
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THIS ISSUE

Over a billion people around the world lack access to safe drinking water and over two billion have little or no sanitation. Do we have the resources — and the will — to provide the water to support a booming population? This issue of *Nature* (see introduction, p. **269** and Editorial, p. **253**) tackles the science, economics and politics of the global water crisis. Climate scientists say that unreliable rains and drier summer soils will become more common: Quirin Schiermeier reports on water strategies for a drier world [p. **270**]. The pressure is on farmers to get maximum crop yields with minimum water use. As Emma Marris reports [p. **273**], the collaboration between plant breeders, agronomists and geneticists to that end has been far from smooth. As the population of India grows, the demand for water keeps rising. Daemon Fairless [p. **278**] investigates an ambitious plan to redistribute the country's water supplies by linking rivers in a vast canal network. Jamie Bartram [Commentary, p. **283**] says it is time to improve the global targets for access to water and sanitation to make them relevant to all. In most countries, crop irrigation accounts for most freshwater use — more than drinking water and domestic consumption — but water use in energy production is catching up fast. Mike Hightower and Suzanne Pierce [Commentary, p. **285**] describe the measures being developed to economize on water use in the energy sector. The need for research into water purification is pressing. In an extensive Review Article [p. **301**], Mark Shannon *et al.* highlight the developing technologies that — it is hoped — can provide our drinking water in the decades ahead. Water is (almost) everywhere, yet physicists still trade theory and counter theory to explain its structure: Phil Ball explains [Essay p. **291**]. And Books & Arts [p. **287**] looks at a documentary on water security, and at art inspired by water's surprising patterns. Go to http://www.nature.com/news/specials/water/index.html for the on-line start-page.

COVER GRAPHIC BY DAVID PARKINS

WIRELESS WORLD In *The Big Switch: Our New Digital Destiny*, Nicholas Carr explores the notion that the fully functional personal computer is virtually dead. Instead we will depend on a diffuse cloud of computing power accessed over the Internet; computing will become a mundane utility like electricity. Our reviewer, John Browning, welcomes Carr's role as an antidote to IT industry hype. But he overdoes the pessimism, says Browning. See for yourself in Carr's book, or on his blog, at roughtype.com. [Books & Arts p. **287**]

BANKING SERVICES The International Stem Cell Forum (ISCF) plans to create a global network of stem cell banks by supporting existing banks and encouraging new facilities. *Nature* interviewed Leszek Borysiewicz, head of the Medical Research Council, which is both an ISCF member and a principal funder for the UK Stem Cell Bank, to find out what cell banks can do that informal exchange of cell lines cannot. [News Q&A p. **263**]

MINORITY REPORT The ups and downs of the Democratic primaries are a reminder that Latinos and African-Americans now make up about a quarter of the US population. As well as distinct voting preferences, these and other smaller minority groups have disease susceptibilities that differ from the population as a whole. In a Special Report, Paul Smaglik investigates an increasing trend: many minority researchers are keen to investigate health problems facing their own communities — against a background of years of flat government funding. [Naturejobs p. **382**]

Methane in a 'hot Jupiter'

Theorists predict the presence of water, methane and carbon monoxide in the atmospheres of 'hot Jupiter' extrasolar planets, and last year, analysis of infrared observations obtained as planet HD 189733b passed in front of its parent star provided indirect evidence for the presence of water. Now near-infrared transmission spectroscopy confirms the presence of water and large amounts of methane on HD 189733b. But not the expected carbon monoxide, perhaps due to photochemical enhancement of methane production or chemical gradients in the atmosphere. [Letter p. **329**; News & Views p. **296**; www.nature.com/podcast]

Victors don't punish

Many theories have been offered to explain the evolution of cooperation in humans. One proposal is that costly punishment can promote cooperation. Everyone benefits on average, the theory goes, despite the cost to those doing the punishing. But most of our interactions are repeated, and in such cases punishment can lead to retaliation. Using a variant of the 'Prisoner's Dilemma' game, Dreber *et al.* find that punishment increases the frequency of cooperation, but not the average payoff. Costly punishments confer no overall advantage to the group. And players who end up with the highest total payoff ('winners') tend not to use punishment, while those with the lowest payoff ('losers') punish most frequently. It seems that costly punishment may not have evolved to promote cooperation, but for some other purpose. [Letter p. **348**; News & Views p. **297**; www.nature.com/podcast]

Spring in their step

In spring, many animals start to become reproductively active. They are generally responding to the longer day lengths at this time of the year, but the molecular pathways that mediate the response are not fully understood. Experiments in the Japanese quail, a well established model for studying photoperiodism, have identified the expression of the thyroid-stimulating hormone thyrotrophin in the pars tuberalis, part of the anterior lobe of the pituitary gland, as a critical event in triggering this photoperiodic response. Two waves of gene expression are involved, the first at about 14 hours after dawn on the first 'long' day, and a second a few hours later. [Article p. **317**; News & Views p. **294**]

Found in translation

Transcriptional control of interferon-mediated gene expression plays a major role in the activation of the innate immune response, but little is known about the role of translational control — the control exerted at the stage at which mRNA is converted into a protein. Colina *et al.* show that in mice lacking the translational repressors 4E-BP1 and 4E-BP2, the threshold for eliciting type-I interferon in response to challenge by various viruses is lowered and virus replication is dramatically suppressed. The 4E-BP repressors appear to act by the synthesis of the protein IRF-7, the master regulator of interferon production. By targeting 4E-BP1 and 4E-BP2 with drugs, it may be possible to boost innate immunity against virus infection. [Article p. **323**]

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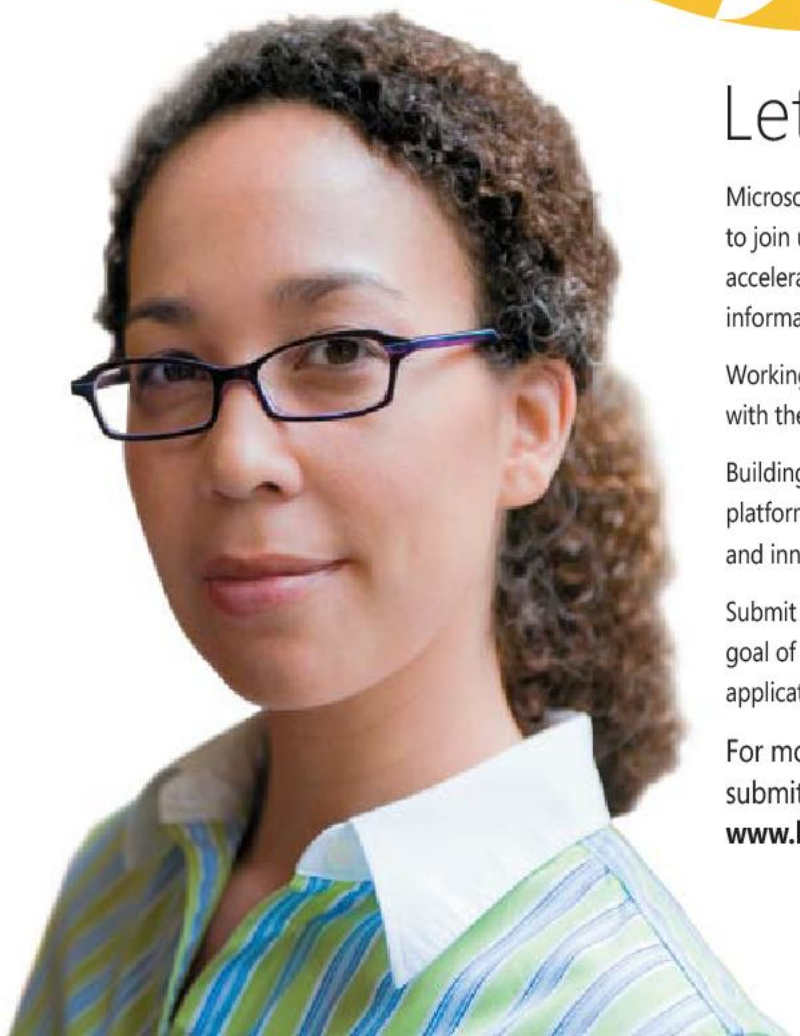

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Eliminating antimatter

The near disappearance of antimatter — predicted to have formed in equal quantities to matter in the Big Bang — is one of the reasons why life can even exist. A prerequisite for understanding the elimination of antimatter is the nonconservation of charge-parity (CP) symmetry. A paper from the Belle Collaboration, a large-scale experiment running at the 'B factory' electron–positron collider at the KEK high-energy physics laboratory in Japan, has tackled this thorny matter of fundamental physics by using 535 million *B* meson/anti-*B* meson pairs to measure CP-violating asymmetries. Previous work had suggested a difference in the decay of charged and neutral particles — an asymmetry in the decay rate of about +7% for charged *B* mesons and −10% for neutral *B* mesons. The new study reduces uncertainty on the charged particle decay rate asymmetry by a factor of 1.7, providing stronger evidence for a large deviation in direct CP violation between charged and neutral *B* meson decays. [Letter p. **332**; News & Views p. **293**]

Inner Solar System

The neodymium–samarium system boasts two useful isotopic clocks. Decay of samarium-147 to neodymium-143 is a mainstay of studies of ancient volcanic rocks. And decay of samarium-146, with a relatively short half life of 103 million years, to neodymium-142, is a high-precision tool used for dating mantle differentiation in planetary bodies. The chronology assumes, however, that the composition of the total planet is identical to that of primitive undifferentiated meteorites called chondrites. The difference in neodymium isotope ratios between chondrites and terrestrial samples may therefore indicate the early isolation of the upper mantle, or a non-chondritic bulk Earth composition. Caro *et al.* present high-precision neodymium isotope data for 16 martian meteorites and show that Mars also has a non-chondritic composition. This suggests that the Earth, Moon and Mars all accreted in part of the inner Solar System with an Sm/Nd ratio about 5% higher than that of material accreted in the asteroid belt, the presumed source of chondrites. [Letter p. **336**]

Living fossils

Stromatolites are living, layered structures formed in shallow waters by a combination of microbial biofilms — usually of blue-green algae — and granular deposits. They are rare today but for about 2 billion years, following their arrival in the fossil record 3.5 billion years ago, they are the main evidence of life on Earth. Modern stromatolites still look like their fossilized forebears. But are the modern microbes remnants of ancient ecosystems or just latecomers following a similar lifestyle? A metagenomic study of the bacteriophage communities in modern stromatolites and thrombolites (like stromatolites but with an irregular internal structure) shows that stromatolite-associated phages are very different from each other and from any other ecosystem studied so far. This finding strengthens the hypothesis that modern stromatolites are remnants of ancient ecosystems. [Letter p. **340**]

An ancient line: stromatolite phages.

Fish numbers: feel the noise

Population sizes of insects, fish, birds and mammals vary greatly from year to year. This variability has often been seen as mere 'noise', an obstacle to unravelling the underlying abundance patterns. This has lead to divergent theories as to whether population growth will decrease at large numbers because of limited resources. Minto *et al.* adopt a novel approach to the problem by focusing on the very patterns of variability that were previously thought of as an obstacle. There should be patterns discernible in the variable data, the thinking goes, if populations are regulated according to their size. The assumption is supported by a study of a global database of fish species. The predictions relate to all population sizes and reveal, in particular, that variance continues to increase the lower the population size becomes. Focusing solely on the average numbers of a population will miss this fact, and increase the risk of numbers plummeting to extinction. [Letter p. **344**]

Reading the mind

Recent functional magnetic resonance imaging (fMRI) studies have shown that, based on patterns of activity evoked by different categories of visual images, it is possible to deduce simple features in the visual scene, or to which category it belongs. Kay *et al.* take this approach a tantalizing step further. Their newly developed decoding method, based on quantitative receptive field models that characterize the relationship between visual stimuli and fMRI activity in early visual areas, can identify with high accuracy which specific natural image an observer saw, even for an image chosen at random from 1,000 distinct images. This prompts the thought that it may soon be possible to decode subjective perceptual experiences such as visual imagery and dreams, an idea previously restricted to the realm of science fiction. [Letter p. **352**; Author page]

Abstractions

LAST AUTHOR

In the quest to understand how the brain processes images, neuroscientists are developing something that sounds like science fiction — a tool for decoding brain activity. Until now, scientists could crudely decode the brain activity that resulted from viewing simple predetermined images, such as faces or places. But on page 352, Jack Gallant, a neuroscientist at the University of California, Berkeley, and his colleagues describe a computational model of the early visual system that can be used to identify natural images being viewed by a person for the first time. Gallant tells *Nature* that it may soon be possible to decode a person's visual experience from brain activity alone.

Is your ultimate goal to build a "brain decoder"?

No, the tool was not the ultimate goal. We want to build a quantitative and predictive model of the brain's visual system, and a brain decoder provides a useful way to test the model.

How were you able to decode novel images?

Our work builds on knowledge gained from more than 50 years of research in many labs. We used a large sample of natural images as stimuli and then constructed a model that links these stimuli to brain responses obtained by functional magnetic resonance imaging — an indicator of brain activity based on blood flow — in the early visual areas of the brain. The model allows us to predict the brain activity in these areas that would be elicited by any arbitrary image. We tested the quality of the model by evaluating the predictions using a separate set of images.

Do you think a model of the entire visual processing circuit will be in place by the time you retire?

I hope so. We know of 30–40 visual areas in the brain, but we currently have good models of how they work for only two of these. It's too early to do meaningful deductive research on visual areas that are essentially unknowns. That's why we use a 'black box' approach — linking random stimuli to brain activity — rather than testing individual hypotheses one at a time.

Do the privacy and ethical issues of 'brain decoding' concern you?

Yes. In science, whenever you learn more about a biological system, you often have to ask if the knowledge gained is worth its potential misuse. A functional model of the brain, our goal, would be a valuable contribution to neuroscience. However, once we have a model, anyone can use it to build a decoder. We're still very far from any potential application, but down the road, ethical and privacy issues must be dealt with. ■

MAKING THE PAPER

Raimund Dutzler

Solving the structure of a ligand-gated ion channel.

The three-dimensional structures of membrane proteins are notoriously difficult to determine. Raimund Dutzler, a biochemist at the University of Zurich, Switzerland, and his graduate student Ricarda Hilf know this only too well: it has taken them two-and-a half years to resolve the structure of a cation-carrying ion channel from the membrane of the bacterium *Erwinia chrysanthemi*. This channel, called ELIC, belongs to a large family of ion channels that also includes neuronal ion channels in animals, and is the first of this type for which such a high-resolution crystal structure has been determined.

The first crystal structure of any ion channel — a potassium ion channel — was produced in 1998 by Roderick MacKinnon, a feat for which he received the 2003 Nobel Prize in Chemistry. Dutzler, who was a postdoc in MacKinnon's lab at the Rockefeller Institute, New York, set his sights on a different type of ion channel that opens only when bound by a particular ligand. These 'ligand-gated ion channels' are made up of five protein subunits and include key players in chemical signalling at neural synapses. The best-known members of the family are the nicotinic acetylcholine receptor, which controls muscle movement, and the γ-aminobutyric acid (GABA) receptor, which is involved in learning and memory.

Dutzler chose the bacterial channel as the simplest and smallest example of this type of channel. It is composed of five identical protein subunits, and a bacterial channel protein should also be easier to produce in large amounts. Even so, the duo encountered many setbacks, but Dutzler credits Hilf with being "incredibly persistent and efficient".

At first they could not produce enough protein to make crystals. "This is usually the end of any crystallography project," says Dutzler. Undaunted, they fused the channel protein to another protein, called maltose-binding protein, to improve production, and obtained crystals. But these first crystals were not of good enough quality to diffract X-rays at the resolution needed. Dutzler and Hilf had to screen many different crystallization conditions before they were able to make crystals that diffracted X-rays at a resolution of 3.3 ångstroms, the resolution required to identify the positions of individual atoms.

And they weren't done yet. To resolve the atomic structure of a protein, one needs several sets of extremely precise X-ray-diffraction measurements in order to construct the electron-density map that is ultimately used to identify atomic positions.

Fortunately, the team had access to a new and highly sensitive X-ray detector called Pilatus at the Swiss Light Source, the synchrotron facility at the Paul Scherrer Institute in Villigen, where the X-ray data were collected. "We had the most optimal infrastructure. Without it, this project could have taken twice as long," says Dutzler.

Dutzler and Hilf believe that the structure that finally emerged (see page 375) is conserved among all pentameric ligand-gated ion channels. "It's the first piece of the puzzle," says Dutzler, adding that they would like to discover what the ligand for this channel is so that they can figure out the mechanics of how it opens to conduct ions. ■

FROM THE BLOGOSPHERE

The recent retraction by Nobel laureate Linda Buck and colleagues of a 2001 *Nature* paper sparked discussions on NPG blogs. On Action Potential, the *Nature Neuroscience* blog (http://tinyurl.com/23bnwg), Debra Speert calls it "the highest profile retraction that I can recall in neuroscience", and on the Nature Network neuroscience forum (http://tinyurl.com/34gxn9) readers are asked for their views on the role of journals and scientists in retracting published work.

The Nature journals correction policy is at http://tinyurl.com/3cluba. For a retraction or other type of correction to be published, all authors typically need to sign it. If some of the authors do not agree, the editors seek advice from peer reviewers and, if necessary, the institution and/or funder. In the event that the retraction or correction is published, the name(s) of the dissenting author(s) are noted in the text of the correction. More information about Buck's retraction is in a News story (*Nature* **452,** 13; 2008), and includes a clarification from one of the paper's authors in the online comment thread. ■

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Vol 452 | Issue no. 7185 | 20 March 2008

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Vol 452 | Issue no. 7185 | 20 March 2008

A fresh approach to water

The water shortage that threatens humanity will have wide-ranging consequences for agriculture and energy production, requiring significant shifts in the way this precious resource is managed.

From space, most of our planet is a deep, satisfying blue. Water, the essential ingredient for life, seems to be everywhere. But, as this issue highlights, the prospects at ground-level are not so agreeable. It is salutary to realize that in our issue of this very date 5 years ago, we wrote an Editorial that, with small amendments, we might well have simply reprinted this week (see *Nature* **422**, 243; 2003). Our planet is facing a water crisis in public health: more than a billion people in developing nations lack access to safe drinking water, and more than 2 billion lack proper sanitation (see page 283). And in the near future, water shortages are likely to spread into other key sectors — notably agriculture (see page 273) and energy (see page 285).

Some of this looming world crisis will be driven by climate pressures, as rising temperatures lead to drier soils and less reliable rainfall (see page 270). But much of it will also be driven by population growth and rapid economic development. As nations such as India and China grow more prosperous, for example, their citizens are switching to more protein-rich Western diets. It takes some 15,500 litres of water to produce a kilogram of industrial beef, ten times as much as is needed to produce 1 kilogram of wheat. These nations are likewise shifting their energy consumption towards intensities common in the developed world. The United States alone is already using more than 500 billion litres of fresh water per day — over 40% of its freshwater withdrawals — for cooling electric power plants. That's roughly the same as the quantity used for irrigation.

The resulting pressures on water supplies are unrelenting. Global energy demand is projected to increase 57% by 2030, and water demand for food production might easily double. By 2050, feeding the world's growing population may require some 12,000 cubic kilometres of water — the volume of Lake Superior — every year. Yet many of the world's rivers and lakes are already dramatically overused: China's Yellow River doesn't always reach the ocean, and Lake Mead in the American southwest could be dry by 2021 if water usage is not curtailed. Such bleak realities have led some countries to contemplate ambitious, and arguably ill-considered, schemes for redirecting their water supplies (see page 278).

"There are many new ideas and fresh approaches that could greatly ease the water crisis."

Shaking off the blues

And yet, the situation is far from hopeless. There are many new ideas and fresh approaches that could greatly ease the water crisis — if only we can collectively figure out how to implement them. In previous decades, for example, water research and policy have focused mostly on the 'blue water' in rivers, lakes, reservoirs and underground aquifers. But blue water accounts for only 40% of the world's freshwater balance, and for much less in dry regions. The key to tackling the crisis in the most food-insecure parts of the world is managing 'green water': the less spectacular, but more abundant moisture that infiltrates the soil from rainfall, and that can be taken up by the roots of plants. Experts estimate that in regions such as sub-Saharan Africa, where more than 95% of crops are rain-fed, only 10–30% of the available rainfall is being used in a productive way. The fixes they suggest are decidedly low-tech: harvesting rainwater, planting roots deeper, better terracing, and switching from ploughing to tilling. Yet the potential gains could be enormous. In heavily irrigated regions such as south Asia, meanwhile, equally simple improvements in water usage could take the pressure off precious blue-water supplies, and hence drinking water.

This emphasis on low-tech agricultural solutions should take nothing away from efforts to develop hardier, more drought-resistant crops through breeding programmes and genetic manipulation. The world is going to need all the solutions it can get. Nonetheless, low-tech efforts can offer big gains at comparatively modest costs. The policy challenge is to figure out who is going to bear those costs, and do the hard, unglamorous work of translating ideas into action. Who, for example, will teach poor farmers how to make better use of their natural resources? And where will they get the financial support to make risky-seeming changes to farming practices in the face of unreliable rains?

A question of control

For the energy sector, meanwhile, there are big gains to be had from water conservation and reuse. Instead of using pristine freshwater, for example, power plants could switch to brackish groundwater or treated wastewater. And this is another arena in which new technologies also have a role (see pages 260 and 301).

Here again, the fundamental challenge is to agree on who is in charge. The two countries doing best in that regard are Israel, where severely limited water supplies have led to a national system in which nearly every drop is recycled; and the Netherlands, where an overabundance of water encroaching from both sea and sky has led to a national strategy to control every aspect of the resource. But these countries are the exceptions, not the rule. More typical is the chaotic situation in the United States, where more than 20 federal agencies deal with some aspect of water — from flooding control to coastal commissions. Water policy is rarely coordinated at a regional or national level, and coherent solutions are almost impossible.

That situation has recently begun to change in the United States, as in the efforts to coordinate water usage in the Colorado River basin. But it has to change everywhere. Unless policy-makers want water resources to be constantly squabbled and fought over, with farmers pitted against city dwellers, upstream users against downstream users, and region against region, every nation needs to think about water strategically. ■

Warning signs

Giving drug firms immunity from prosecution over inaccurate labelling would not serve the public.

In April 2000, at a Vermont health centre, Diana Levine was injected with the anti-nausea drug Phenergan in what was supposed to be a vein, but was in fact an artery. The drug caused arterial spasms, which led to gangrene; in the end, Levine's hand and forearm were amputated. Arterial spasming was a known danger with this drug, which was labelled with a warning approved by the US Food and Drug Administration (FDA). Nonetheless, Levine sued Wyeth, Phenergan's maker, in Vermont state court, alleging that the labelling was inadequate. The jury agreed, and the court awarded Levine $6.8 million in damages. Wyeth has now appealed the verdict all the way to the US Supreme Court, which recently agreed to hear the case in October.

The question before the court is whether the FDA's seal of approval on a drug's label should preempt a plaintiff's right to sue the maker on the basis of labelling inadequacies. Such 'failure to warn' claims underpin the vast majority of drug-injury lawsuits. The drug companies maintain that the assessment of FDA experts, who carefully weigh the risks and benefits of a drug before approving its label, should trump the findings of inexpert juries confronted with grievously injured plaintiffs.

The companies have a point. Frivolous or misguided litigation can doom drugs that might have benefited millions. The cost of bringing a new drug to market averages as much as $1.7 billion by one estimate, not least because the companies have to meet a multiplicity of FDA requirements along the way. Fairness suggests that clearing all those hurdles should earn them at least some degree of legal protection — as long as they have shown due diligence in testing their drugs, and have openly declared all the relevant data, both pro and con. Nonetheless, 'some degree' is different from blanket immunity, which the court should avoid for at least two reasons.

First, the industry's argument wrongly implies that science can identify all the risks in advance with absolute certainty. FDA drug approvals are based on clinical trials that are necessarily limited in size and duration, so harmful side effects sometimes won't emerge until a drug is in broader use. Witness the painkiller Vioxx, which was taken by tens of millions of people before it was found to increase their risk of heart attacks and strokes. Removing the right to sue when new side effects emerge would rob most of those affected in the future of redress for their injuries.

Second, the industry's argument presumes that the FDA is poised to update warning labels at a moment's notice. As former FDA commissioner David Kessler said recently, this notion is "unrealistic". With 11,000 drugs on the US market, and nearly 100 more approved each year, the agency is overwhelmed trying to keep up with new side effects — making lawsuits in state courts an important complement to its regulatory efforts. And, as Kessler also noted, the threat of potential litigation helps to ensure that drug firms are prompt in reporting new adverse effects to the agency and working to update labels.

Supreme Court decisions are notoriously difficult to predict. But it is worth noting that in a recent decision that affects the most advanced, expensive and — in some cases — risky FDA-approved medical devices, the court granted manufacturers exactly the kind of immunity the drug industry is seeking in *Wyeth* v. *Levine*. To extend such broad-ranging protection to drugs would be both ill-advised and unjust. Whatever the justices decide, they must preserve the right of future Diana Levines to have their day in court. ■

The EIT farce

Universities should target the challenges that a virtual technology powerhouse probably won't meet.

When the European Parliament approved the controversial European Institute of Innovation and Technology on 11 March, one dissenting parliamentarian complained that the "initiative has degenerated into a farce". He judged it "poorly defined and under-funded". He was right.

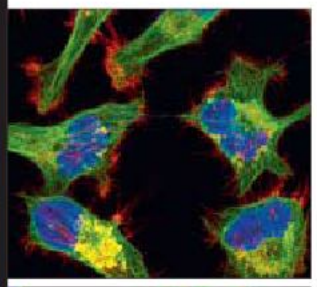
The original 2005 concept was to recreate the Massachusetts Institute of Technology (MIT) in Europe, where research in academic institutions fails to transfer to industry efficiently. But as few believed that such an institute could be created in a top-down fashion, and European Union (EU) member states were unwilling to provide major institutional investment, the 'EIT' concept evolved into just a small headquarters. This will select and administer distributed networks of academic institutes and private companies focusing on problems considered most pressing in Europe, such as renewable energy. In the same stroke, the European Parliament inserted the word 'innovation' into the EIT's name, although the original acronym lives on.

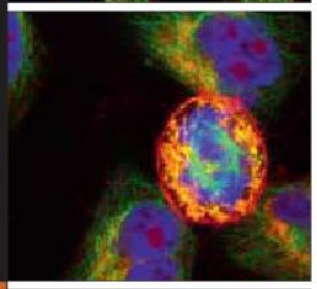
The result is an enfeebled EIT that is mismatched with the problems it is designed to solve. The EU has set aside just €300 million (US$475 million) for it in the first six years, a fraction of the minimum of €2.3 billion considered necessary to fulfil the EIT's purpose. It is relying on industry and other sources to make up the difference.

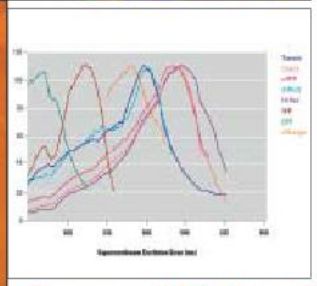
That seems unlikely. But whatever the outcome, the very existence of the EIT concept — and its survival through the rough seas of EU politics — is an indictment of Europe's suffocating national bureaucracies, which have made it impossible for universities and publicly funded research institutes to evolve into MITs on their own. 'Elite' has too often been treated as a dirty word, and interactions with industry considered a betrayal of academic purity. In many countries, including France, Germany and Italy, it is still generally impossible to offer internationally competitive packages to top researchers.

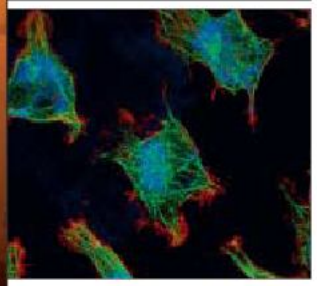
But a belated recognition of the need for change is now taking hold. Important steps have been taken in most countries to develop appropriate legal frameworks and infrastructures for technology transfer. In 2003, both Germany and Italy suggested founding their own 'MITs' — precipitating the same political furore seen later when the EIT was proposed. The Italian Institute of Technology was finally realized in Genoa. In Germany, the idea was quickly abandoned in favour of a plan to encourage existing universities to excel in an MIT-like way. Its competition to award winners the 'élite' title has been a success.

The EIT may yet surprise its critics. Either way, national efforts to boost universities are by far the best way to address the problems that the EIT is intended to solve. ■









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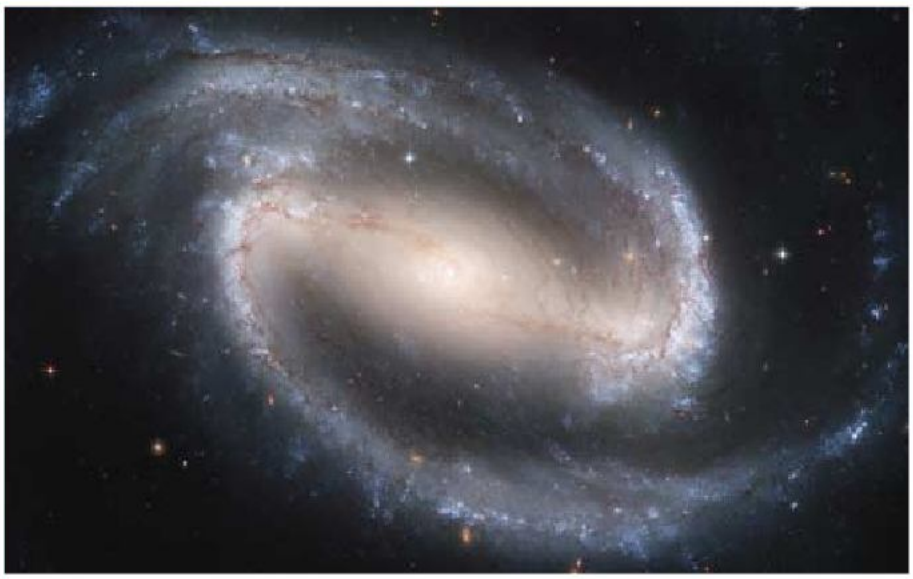

NASA/ESA/HUBBLE HERITAGE TEAM

Star walks into a bar

Astrophys. J. **675**, 1141; 2008

Spiral galaxies with star-dense 'bars' in their centre are commonplace in the present-day Universe, but were not in the past.

Kartik Sheth at the California Institute of Technology in Pasadena and his colleagues studied over 2,000 spiral galaxies from different cosmic epochs imaged by the Hubble Space Telescope.

The survey shows that bar galaxies make up roughly 65% of spiral galaxies formed in the very recent past, but were only a third as common when the Universe was half its present age. The bars are frequently found in lower-mass galaxies, whose stars form later on average than those in higher-mass systems.

ECOLOGY

Chain of death

Am. Nat. doi:10.1086/587068 (2008)

Loss of a species can spark a cascade of secondary extinctions as the community adapts to its absence. To determine the impact of such secondary extinctions on biodiversity, Owen Petchey of the University of Sheffield, UK, and his colleagues used computer models of food webs and field data.

The impact of secondary extinctions on reducing the diversity of feeding habits in a community turned out to be larger than expected from chance. Secondary extinctions were also more common among species with rather unique feeding habits.

Such correlations could be used to identify high-risk species whose extinction would trigger large losses of biodiversity.

CHEMISTRY

Pole-dancing pumpkins

Angew. Chem. Int. Ed. doi:10.1002/anie.200705211 (2008)

A molecular valve that is triggered by changes in pH and works in a watery environment like that in the human body has been developed by Jeffrey Zink and his colleagues at the University of California, Los Angeles. The valves are on porous silica nanospheres studded with pole-like molecules. Each 'pole' is threaded with a large pumpkin-shaped molecule of cucurbituril. At neutral pH, the cucurbiturils sit at the bottom of their poles, blocking the pores. As the pH increases, they move up the poles, unblocking the pores and releasing the contents of the sphere, which could be a small-molecule drug. With tweaks, the system could respond to very small differences in pH such as those between healthy and diseased cells.

EVOLUTION

Royal machinations

Proc. Natl Acad. Sci. USA doi:10.1073/pnas.0710262105 (2008)

Ant societies seem to be models of cooperation; queens monopolize reproduction and are maintained by sterile female workers. But William Hughes and Jacobus Boomsma at the University of Copenhagen, Denmark, have found a few cheaters among the leaf-cutter ants (*Acromyrmex echinatior*).

They found that a queen's offspring by certain males were much more likely to develop into queens themselves — hogging the reproductive opportunities. Cheats make up no more than 20% of the male lines. Evolutionary theory says cheaters must be rare in order to prosper or cooperators will be selected to suppress them.

MARINE ECOLOGY

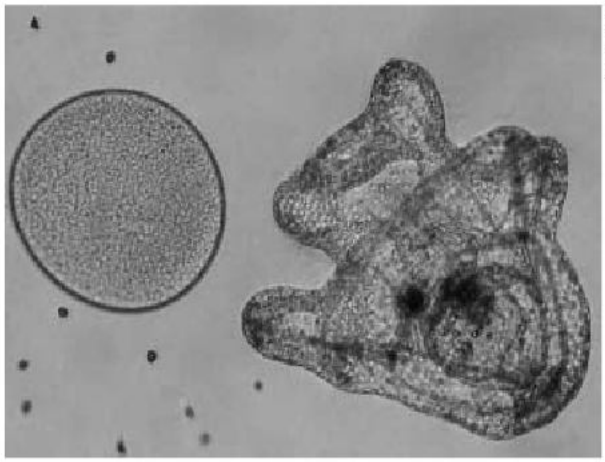
Defensive cloning

Science doi:10.1126/science.1151995 (2008)

Larvae of the sand dollar (*Dendraster excentricus*) clone themselves as a defence mechanism when they detect the presence of predatory fish.

Dawn Vaughn and Richard Strathmann at the University of Washington in Seattle exposed 4-day-old sand dollar larvae to mucus from fish. Within 24 hours the larvae had formed clones by budding and fission. The clones are smaller than the original larva (clone pictured below with an egg for comparison), which probably helps them avoid being eaten.

Until now, cloning was not considered a defensive strategy. Echinoderm larvae were only known to clone themselves in response to abundant food or temperatures favourable to growth and reproduction.


D. VAUGHN

ASTRONOMY

Good samarium

Astrophys. J. **675**, 723–745 (2008)

Rare-earth elements spotted by astronomers are helping pin down how distant stars burn.

'Heavy' elements — anything heavier than iron — can be created through one of two fusion pathways. To find out which process rules where, astronomers led by Ian Roederer at the University of Texas at Austin collected spectra from two stars suspected

of using different pathways, and scrutinized the elements europium, samarium and neodymium. The team was able to count the fraction of isotopes of each element, concluding that one process dominated in one star and the alternative in the other star.

While not surprising, the finding happily suggests that scientists' understanding of nucleosynthesis "is not wildly mistaken", the authors write.

ANIMAL BEHAVIOUR

Coming out of their shells

Proc. R. Soc. Lond. B doi:10.1098/rspb.2008.0025 (2008)

Mark Briffa and colleagues at the University of Plymouth, UK, have detected the first signs of personality among crustaceans.

Briffa's team devised a new statistical method to differentiate between natural variability in responses of individual European hermit crabs (*Pagurus bernhardus*, pictured right) to different situations, and a consistent trend in responses that reflects a 'personality'.

P. bernhardus retreats into its commandeered shell at any sign of danger. The team timed how long crabs took to re-emerge from their shells in a variety of situations. Some bold crabs consistently emerged sooner than others.

NANOTECHNOLOGY

Cells on a roll

Nano Lett. doi:10.1021/nl073322a (2008)

Sorting cells in the lab generally means labelling them with chemicals and using expensive cell-sorting equipment. Robert Langer and collaborators at the Massachusetts Institute of Technology have instead capitalized on the natural proclivity of some cells to roll along surfaces, including the insides of blood vessels, to sort them. On coating part of a flow chamber with a ligand called P-selectin, Langer and his colleagues found that cells with the right receptors rolled with the flow until they reached the edge of the coating. The cells then continued to roll along this edge, diverting from the direction of flow at angles of up to 8.6°.

ECOLOGY

Egregious edge effects

Proc. Natl Acad. Sci. USA doi:10.1073/pnas.0800460105 (2008)

How wide is a forest edge? For a fifth of the beetle species found in New Zealand, 'edge effects' — the suite of differences that characterize the fringes of a forest fragment, including increased light and wind — influence the abundance of species more than 250 metres inside the boundary of the forest.

Robert Ewers of Imperial College London and Raphael Didham of the University of Canterbury, Christchurch, New Zealand, also found that edge effects penetrated a full kilometre into the forest for one in eight of a number of common beetle species, a much greater distance than previously reported for invertebrates.

J. GREENFIELD/IMAGEQUEST3D.COM

CHEMISTRY

Cobalt coupling

J. Am. Chem. Soc. doi:10.1021/ja800738d (2008)

Chemists have found a new way to carry out the most basic, but often the most challenging, of chemical reactions — making carbon–carbon bonds. Robert Bergman and his colleagues at the University of California, Berkeley, have developed a nitrogen-containing cobalt reaction system that makes otherwise docile carbon–hydrogen bonds reactive. Cobalt-containing molecules are first reacted with nitric oxide. The resulting complex holds on to a target molecule — a simple alkene with a carbon–hydrogen bond — while this reacts with another similar alkene. The two molecules become joined by a brand new carbon–carbon bond. Chemists trying to make complex drug molecules now have another trick to try.

JOURNAL CLUB

Masayuki Inoue
Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan

A synthetic chemist takes inspiration from sketching structures.

I enjoy drawing chemical structures of complex natural products and imagining how their polar functional groups, such as –OH and –NH_2, interact with biopolymers. I usually first draw a carbon framework of the molecule on paper and then add the required groups. Of course, this order of 'functionalizations' has almost nothing to do with any synthetic scheme I might use for that molecule. Tedious multi-step manipulations are often needed just to introduce one oxygen or nitrogen. Making a molecule will never be as easy as drawing one.

Many research groups are trying to make it easier by devising one-step introductions of complete polar groups into carbon frameworks. One of the latest examples comes from Mark Chen and Christina White (*Science*, **318**, 783–787; 2007). They used a new iron catalyst and hydrogen peroxide to convert specific hydrogens to hydroxyl groups on the carbon skeletons of a variety of molecules.

The catalyst seems to be able to differentiate a site of functionalization from other potentially oxidizable C–H bonds by the balance of two factors: electron-richness and steric accessibility of the bond. Chen and White were able to oxidize the antimalarial natural product (+)-artemisinin at just one predicted position to produce (+)-10β-hydroxyartemisinin. Their work represents a definite advance in the direct functionalization of carbon skeletons.

Every chemist dreams about placing functional groups anywhere they want as easily as drawing them on paper. The direct C–H oxidation reaction should allow us to perform such manipulations and holds great promise for simplifying the synthesis of complex molecules.

Discuss this paper at **http://blogs.nature.com/nature/journalclub**

NEWS

222 NIH grants: 22 researchers

A whopping 200 scientists received six or more grants each from the US National Institutes of Health (NIH) in 2007, according to data analysed by *Nature*. One principal investigator was awarded 32 grants, the data reveal, and many others got eight or nine.

The amounts awarded to some of these grandee grantees held some surprises too. Robert Sherwin of Yale University in Connecticut received eight grants totalling US$14.5 million last year for his research into diabetes; Harold Varmus, president of the Memorial Sloan-Kettering Cancer Center, New York, received grants of $13 million for work on cancer; and cell-death researcher John Reed of the California-based Burnham Institute for Medical Research received nearly $11 million in 11 grants.

The data that *Nature* analysed include all types of NIH grant, including supplemental grants and small grants awarded to organize conferences or run training workshops. Closer inspection reveals that some researchers received a wealth of grants for precisely these reasons — Andrew Robertson, the recipient of the 32 grants, is a conference organizer for Keystone Symposia, which necessarily requires him to juggle multiple projects. His grants average out at $15,300 each. But the multiple grants supporting some other investigators are not as immediately explicable.

Last month, advisory panels reviewing the NIH peer-review system recommended that researchers should devote at least 20% of their time to any project awarded a research grant (see *Nature* **451**, 1035; 2008). This would limit the number of grants awarded per investigator to five. "Are you really able to sustain the research if you have five or ten grants?" asked NIH director Elias Zerhouni after a congressional hearing on 5 March. "If you are going to be a principal investigator on a grant, you have to give the time." Zerhouni told *Nature* he wants to place a limit on the number of grants that researchers can get each year.

"The absolute number of grants is misleading," says Sten Vermund, director of the Institute for Global Health at Vanderbilt University Medical Center in Tennessee. He received 11 grants worth $24 million in 2007, but most of that was a single $19-million grant to manage a global HIV-prevention trial involving hundreds of researchers working on four continents at dozens of institutions. Seven of his grants were smaller and all essentially awarded for the same thing: an international AIDS training programme. Vermund acknowledges that a former stint at the NIH overseeing a $50-million grant portfolio in AIDS vaccine trials taught him a lot about how successful grant applications are packaged and marketed. "I don't want to make myself sound like a grant-writing technician, but let's be honest: that is a nontrivial part of success in biomedical research."

Other researchers seem to run 'labs-on-steroids', earning multiple grants through the sheer volume and quality of their work (see 'Day in the life of an 11-grant grandee'). They argue that if they're willing to work longer and harder — and still produce top research — then so be it. "Different people can achieve different things in 20% of their time. You should always reward the best science," says David Rawlings, the 51-year-old director of the Research Center for Immunity and Immunotherapies at the University of Washington in Seattle. He was supported last year by nine NIH grants worth $3 million.

Joann Boughman, executive vice-president of the American Society of Human Genetics, says that the NIH needs to keep young researchers working in independent situations so that they can have 'eureka' moments. At the same time, she says, the agency needs to support established laboratories that produce streams of rich data, often leading to new experiments — and new grants. "The question is, do the rich get richer while the poor get poorer?" she asks.

The reality is that grantees like Vermund inhabit a different world from the vast majority of biomedical scientists. Frozen funding at the NIH is creating an environment of "anxiety and fear" where talented young researchers repeatedly have their grant proposals rejected.

Zerhouni says that the inequities between the haves and have-nots were caused by a doubling of NIH funding between 1998 and 2003. As funding levels rose, many new PhD positions were created. Established investigators, using data produced by the new PhDs, were able to submit better grant proposals. But hordes of these grant-hungry PhDs were left standing when NIH funding flattened out after 2003. The agency now funds significantly more people over the age of 70 than under the age of 30. "We're eating our seedcorn," says Zerhouni.

Changes to the NIH peer-review system will be unveiled in mid-April. ■

Eric Hand with additional reporting by Meredith Wadman

SCIENTISTS SUPPORTED BY EIGHT OR MORE NIH GRANTS IN 2007

Principal investigator	Institution	Research type	Number of grants	Total value (US$1,000)
Andrew Robertson	Keystone Symposia	Conference organizer	32	490
Terri Grodzicker	Cold Spring Harbor Laboratory	Conference organizer	16	869
Sten Vermund	Family Health International	HIV-prevention trials	11	24,132
John Reed	Burnham Institute for Medical Research	Cell death	11	10,868
Jeffrey Murray	University of Iowa	Birth defects	11	7,060
Joseph McCune	University of California, San Francisco	Translational science	9	25,396
Bert O'Malley	Baylor College of Medicine	Reproductive biology	9	8,229
David Rawlings	Children's Hospital, Seattle	Gene therapy	9	3,000
David Allison	University of Alabama at Birmingham	Obesity	9	2,499
David Stewart	Cold Spring Harbor Laboratory	Conference organizer	9	255
Robert Sherwin	Yale University	Diabetes	8	14,550
Harold Varmus	Memorial Sloan-Kettering Cancer Center	Cancer	8	13,119
Pamela Davis	Case Western Reserve University	Cystic fibrosis	8	12,518
Bruce Rosen	Massachusetts General Hospital	Brain imaging	8	9,063
John Tainer	Scripps Research Institute	DNA biochemistry	8	5,375
Eric Nestler	University of Texas Southwestern Medical Center	Drug abuse	8	5,147
Jennifer Grandis	University of Pittsburgh	Head and neck cancer	8	3,702
Richard Chaisson	Johns Hopkins University	HIV, tuberculosis	8	3,277
William Petri	University of Virginia	Intestinal parasites	8	2,993
Joseph Vinetz	University of California, San Diego	Infectious diseases	8	2,758
Victor Garcia-Martinez	University of Texas Southwestern Medical Center	HIV transmission	8	2,463
Cun-Yu Wang	University of California, Los Angeles	Cancer	8	1,671

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www.nature.com/news

Day in the life of an 11-grant grandee

John Reed's workday begins when he wakes up at 3 a.m. to write grants and papers. At 6 or 6:30, he goes for a long run, swim or bicycle ride. By his own count, he has run 20 half-marathons and 10 marathons. Now aged 49, he's into half-triathlons — but only because full triathlons take too much training time.

By 7:30, Reed is at work at the Burnham Institute for Medical Research in La Jolla, California, juggling roles as its president and director of a 35-person lab that specializes in cell death. If his researchers need him urgently, they e-mail a secret address that goes directly to him. They each have weekly goals that must be met, and progress is measured with project-management software. "It's a no-nonsense, get-the-job-done, focused environment," Reed says.

On many evenings he has business dinners and meetings to attend. Every other evening, he goes home to spend time with his family. At weekends, he sleeps in — until 4:30 a.m.

Kenneth Yip, a 28-year-old postdoctoral researcher from Canada, joined the lab two years ago knowing that working with Reed would help him to build an attractive CV. "I don't know many principal investigators at that level who have washboard abs," Yip says. "He does everything 100% — which is 200% for the rest of us."

Reed's results speak for themselves. Between 1995 and 2005, he was the most highly cited author in all of cell biology, with 23,729 citations, according to Thomson's ESI ranking. His lab has averaged one paper per person per year.

His productivity has been rewarded with support from the US National Institutes of Health (NIH). In 2007, he received 11 NIH grants worth almost $11 million. He says he deserves them all. And he doesn't support a cap to the number of grants permitted per researcher. "The evidence is that some labs and some people can handle a larger portfolio," Reed says. "I don't think we should apply a one-size-fits-all mentality." **E.H.**

The Solar System's first breath

HOUSTON, TEXAS

Scientists have made the crucial measurement of oxygen composition at the birth of the Solar System. The discovery fulfils the top science priority of the NASA Genesis probe, which slammed into the Utah desert in 2004 on its return to Earth when its parachute failed to open.

"Despite crashing, all the major science objectives of Genesis will be met," says Kevin McKeegan, a cosmochemist at the University of California, Los Angeles. He announced the finding on 10 March at the Lunar and Planetary Science Conference in Houston, Texas.

The finding that the Sun is relatively richer than Earth in oxygen-16, the most common oxygen isotope, contradicts the conventional wisdom that Earth has the same oxygen isotope composition as the Sun. The discovery also gives researchers a reference point for the oxygen composition at the origin of the Solar System. Genesis trapped the stream of ionized particles known as the solar wind — which, because it emanates from the relatively unchanged outer layers of the Sun, is thought to carry primordial oxygen among its elements.

Oxygen-16, with eight protons and eight neutrons, comprises 99.8% of the oxygen on Earth. There are smaller amounts of oxygen-17 and oxygen-18, whose proportions vary throughout the Solar System. Scientists have measured slightly different proportions on Earth, Mars, the Moon and in meteorites, as if each place has its own oxygen fingerprint. "We had a map for oxygen isotopes," says McKeegan. "But we didn't know which way was up."

Researchers have gone to great lengths to try to discover the original proportion of oxygen isotopes in the Sun. Two rival groups published contradictory results from analyses of lunar soils, which are thought to contain embedded solar oxygen as the Moon lacks an atmospheric shield against the solar wind (see *Nature* 440, 751–752; 2006). One of those researchers, Marc Chaussidon, is pleased that the new findings could settle the debate.

"There has been this question for years," says Chaussidon, a cosmochemist at the Research Centre for Petrochemistry and Geochemistry in Nancy, France. "Everybody would have bet that the Sun had the same composition as Earth and the meteorites. In fact, Earth is not like the Sun."

The result represents a triumph for the Genesis scientists, who have salvaged what they could from the wreck, including isotopic analyses of noble gases (A. Meshik *et al. Science* 318, 433–435; 2007). But oxygen is tougher to measure, as it is so plentiful and reactive. McKeegan and his group used a mass spectrometer on a 3-millimetre-square section of a silicon wafer containing oxygen from the solar wind.

Using a beam of caesium ions, the researchers eroded the top 20 nanometres of the sample to remove any contamination by Earth-based oxygen. Then, in a vacuum, they measured the isotopic composition of the Sun's oxygen, using the ion beam to knock the atoms loose from the silicon trap, and found a greater proportion of oxygen-16 than on Earth.

The result raises more questions, says Chaussidon. Now, scientists need to understand why Earth's oxygen composition is different from the Sun's, and what chemical processes caused the change. Whatever the process, it would have sucked out oxygen-16 while the gas of the proto-Solar System condensed into solid grains that coalesced into the planets.

It would also have been one of the very first things to happen in the 4.5685-billion-year-old Solar System. Chaussidon says the mystery process would have stripped away the oxygen-16 within the system's first few million years of existence. ■

Eric Hand

NASA JOHNSON SPACE CENTER

Genesis' collectors trapped atoms in the solar wind.

SPECIAL REPORT

Purification with a pinch of salt

Climate change, growing populations and political concerns are prompting governments and investors from California to China to take a fresh look at desalination. **Quirin Schiermeier** wades in.

Water has always been a volatile topic in Australia, the world's driest inhabited continent, but the political row that broke out last week was perhaps surprising. Protesters are complaining that a planned desalination facility outside Melbourne, Victoria, will generate too much freshwater.

The US$3-billion government-owned plant will produce more than 300,000 cubic metres of drinkable water a day when it opens in 2011, putting it among the world's biggest. Environmental groups claim that the plant is unnecessary. Even if water consumption rose by 25%, there would be an excess of about 60% in supply over consumption by 2016, according to Neil Rankine, a spokesman for protest group Your Water Your Say. Rankine's figures are based on the state increasing other efforts such as recycling water and harvesting rainwater.

Nobody, of course, is actually worried about the possibility of having too much water — at issue is the cost to the environment. "Desalination is the most energy-intensive form of water supply," says Peter Gleick, president of the Pacific Institute, an independent environmental think-tank in Oakland, California. The Victorian plant will sit next to a six-turbine wind farm, but few believe that the small, inefficient farm will be able to power the huge facility. The highly concentrated brine discharged by the desalination processes is also of ecological concern.

The economic payout is steep too. Unlike the mass production of other consumer goods, there is no pronounced economy of scale at play in 'making' water — even massive plants cannot produce desalinated water at significantly lower costs than small, community-based facilities.

Increasingly, countries are willing to pay the price. Nations from Australia to Britain, the United States to China, have desalination projects in the works — 75 major plants are at various stages of development globally (see graph, below). Currently, more than 40 million cubic metres of desalinated water are produced every day by 15,000 or so production facilities worldwide. "In the next 10–20 years we will see a massive increase in capacity and production," says Bruce Durham, an independent consultant who has worked with the water industry for more than 30 years. In California alone, proposals have been put forward for at least 20 new large desalination facilities (see map), which together could ultimately supply some 6% of the state's urban water demand.

Costs have come down. Even the very energy-intensive thermal plants in the Gulf region — which purify seawater by boiling and condensing — can produce fresh water at less than US$1 per cubic metre. And the desalination plant at Ashkelon in Israel, once the world's largest, produces more than 300,000 cubic metres of freshwater per day at costs of around 50 cents per cubic metre. That's 1,000 litres of drinking water for less than half the retail price of a 1-litre bottle of Evian. But on average, the technique is 3.5 times more expensive than using other sources of freshwater such as pumping from aquifers.

Technological future

Advances in chemical engineering promise to make desalination more affordable. Polyamide membranes are the basic components of reverse-osmosis plants, which produce more than half of the world's desalinated water and are replacing less-efficient thermal distillation facilities. To remove dissolved organic matter and other impurities, brackish water or seawater is pre-filtered and then forced under pressure through bundles of these semi-permeable membranes, which separate salts from the water (see Fig. 4, page 307).

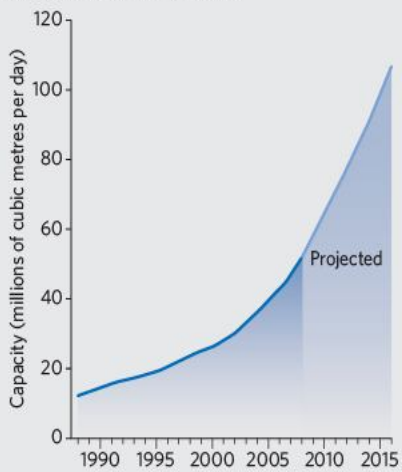
Pretreatment cannot fully prevent the membranes from fouling and degrading, so they need to be cleaned chemically and replaced frequently — a major cost factor. Every company has its own way to fight 'bio-fouling', salt deposition and other processes that reduce the flux of water through the membranes.

In a bid to tackle fouling, where geology allows it, some operators of coastal plants have begun to draw water from beach wells rather than from the open sea. The sand acts as a natural filter, pretreating the seawater. Beach wells also have the advantage of preventing fish and marine life from getting trapped and killed in the uptake pipes, a widespread problem with coastal desalination.

But although polymer membranes have become more permeable and durable since they were first developed, neither the basic technology used in reverse osmosis nor the membrane materials used in the desalination process have changed much. Scientists in Singapore — which has recently earmarked US$250 million for developing desalination technologies — are testing alternative techniques such as membrane distillation, which combines both membrane technology and evaporation processing in one unit. This can

PROPOSED DESALINATION PLANTS IN CALIFORNIA

GLOBAL PRODUCTION OF DESALINATED WATER



SOURCE: GLOBAL WATER INTELLIGENCE

WATER SPECIAL
Find all our content on water management online
www.nature.com/news/specials/water/index.html

Desalination plants in coastal Spain use sand as a natural filter to pretreat the seawater.

F. GIERTH/DPA/CORBIS

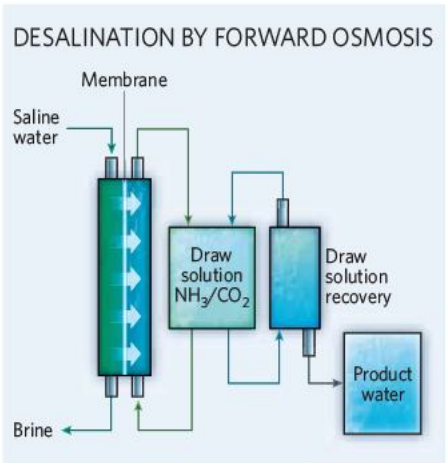
then be coupled with solar energy, geothermal energy or waste heat.

Another promising method is the use of aligned carbon nanotubes — molecular-scale pipettes through which water can be forced frictionless 1,000 times faster than through polymeric membranes. However, no one has as yet demonstrated the desalination ability of nanotubes, or suggested how to get around the fouling problem. Moreover, this technology, which requires hydraulic pressure, would reduce energy consumption by just 20% according to experts.

Prototypes now exist for a desalination technology based on 'forward osmosis', which works at very low pressure. Menachem Elimelech, an environmental engineer at Yale University in New Haven, Connecticut, leads a team that has constructed a pilot desalination plant that uses osmotic, rather than hydraulic, pressure (see graphic, above). The researchers position a concentrated solution of dissolved ammonia and carbon dioxide gases behind a membrane, creating osmotic pressure. This draws the saltwater on the other side through the membrane. Freshwater can then be recovered from the draw solution by heating it to 58 °C so that ammonia and carbon dioxide bubble out of solution and are captured.

"In absolute terms the process is not quite as efficient as reverse osmosis, but the nice thing is that you can use waste heat to decompose salts from solution," says Elimelech.

Besides being less energy-intensive, forward osmosis would greatly reduce brine discharge. Residual brine from existing desalination processes must be watered down to concentrations that are harmless to marine life.



However, forward osmosis requires membranes that must be extremely thin and porous, and tolerant to strongly basic water, and such devices are not yet commercially available, Elimelech says.

Energy will always remain the crucial constraint. Twenty years ago, 5–10 kilowatt hours of electricity was needed to produce one cubic metre of desalinated water. Modern reverse-osmosis plants, such as that at Ashkelon, now need around 2 kilowatt hours to produce the same volume. The world record, achieved in a pilot plant in California, is 1.58 kilowatt hours. The laws of thermodynamics impose a theoretical limit of around 0.7 kilowatt hours on the energy-efficiency of desalination. And because the desired high flux rates require extra energy, plants such as Ashkelon are already close to what is realistically feasible.

"You can further improve membrane materials and you can optimize energy-recovery devices," says Gary Amy, a desalination expert at the United Nations Educational, Scientific and Cultural Organization's Institute for Water Education in Delft in the Netherlands. "But no matter what you try, the energy-efficiency of desalination will soon reach a plateau."

Despite these limitations, well-designed desalination plants can still be more efficient and environmentally sound than large dams, pipelines or canals. "Desalination is one technology that can mitigate the problem of water shortages. The solution it is not," says Mark Shannon, a mechanical engineer at the University of Illinois at Urbana-Champaign, who oversees a science and technology centre for water purification funded by the US National Science Foundation.

As Rankine and his supporters gear up for a new round of protests, Melbourne could do worse than look west to the city of Perth. Its US$329-million desalination plant, which opened in 2006, has won grudging approval. In fact, a second, US$811-million plant is now planned. The secret: renewable energy — the power comes mainly from a wind farm, and up to 90% of it can be recycled by energy-recovery devices. ■

SOURCE: MEMBRANE TECHNOLOGY

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NW125849R

Q&A

Banking on the future of stem cells

Representatives of 21 stem-cell funding agencies from 19 countries — members of the International Stem Cell Forum — met in San Francisco at the end of February to discuss collaborations and how to coordinate cell banks and registries. Among them was **Leszek Borysiewicz**, head of the UK Medical Research Council (MRC), who spoke to *Nature* about the effort.

Researchers already share cell lines, so why are stem-cell banks important?
They make material available to investigators in a characterized way, so that you get reproducibility. You can imagine how important that's going to become as we start to get closer to putting this material back into patients.

So far, there are very few stem-cell banks. How many does the world need?
It is very important to set up several of these banks around the world. We've got to make sure that we coordinate so that the material we are providing is similar. At the end of the day, there will be a balance in terms of how many banks are necessary. When is the redundancy going to be too much and where is the duplication actually productive?

It's odd that, because of the political situation in the United States, the forum includes three members from that nation, but just one from the other countries.
Most of the organizations I sit with are international. The importance is whether the funders themselves wish to commit to an area. It's better to hear what they're doing than to be petty about numbers.

Is the funding situation simpler in the United Kingdom?
In the United Kingdom we have support from central government for stem-cell-related research. So, my belief is that simplifying the process by which investigators can assess resources — rather than putting it into many small compartments — makes it much easier for them. The MRC doesn't earmark funds. If we get high-quality applications, then within the MRC we have the ability to ensure that we make additional budgets available.

Britain is greatly expanding funds for stem-cell research — £17 million (US$34 million) over the next 3 years — but it is still dwarfed by the amount that California is spending.
The fact that California is making the funds available is a great thing. It does put pressure on the United Kingdom to make sure that resources are available for UK researchers, but that's a very positive pressure.

The publicly funded MRC is involved in corporate partnerships. How does that work?
Of course there are checks and balances. It's about ensuring the academic freedom of investigators to work with compounds in a way that they've got freedom to publish and make sure that the information gets into the public domain, and that the collaboration accelerates a push-through into clinical practice.

MRC

What challenges are there in getting stem cells into the clinic?
One of the questions that's going to have to be addressed, particularly for the induced pluripotency [adult cells that have been reprogrammed to an embryonic-like state using genes] lines is the issue of tumorigenicity — knowing how and when you're going to need to activate particular genes, some of which may be predisposed for the development of tumours. These are very real issues that we will have to address as we move forward.

What you don't want is to suddenly get a block that you didn't identify. There's nothing worse than doing a whole lot of basic science on a cell line and then finding that you can't use it to get to the point of a therapy.

As the MRC's head, how do you feel about having to hand money made through technology transfer back to the Treasury?
Do I like having to hand back close to two hundred million dollars? No, of course I don't. But what has to be remembered is that this money is intended for science, so if we're able to deliver the science through resources made available from other sources, that's fine by me. ■

Interview by Monya Baker.

For more on the international forum and the latest developments, visit www.nature.com/stemcells.

R. LEWIS/AP

Safe deposit: researchers hope that stem-cell banks will enhance the reproducibility of their work.

RONALD GRANT ARCHIVE

SHOWBIZ NEWS

Hard-hitting research
Martial-arts legend Jackie Chan has opened a science-education centre to showcase medical research at the Australian National University, located in Canberra, where his family moved in 1962 and he was first nicknamed Jackie. Let's hope the centre gives public science a healthy kick.

WORDWATCH

Nom de simplicité?
"From today, we're known simply as IUCN," trumpets a press release from the organization once confusingly known as IUCN: The World Conservation Union. Handily pasted underneath is a French translation describing how the organization will henceforth be known simply as UICN ...

NUMBER CRUNCH

5 micrometres is the diameter of the world's smallest diamond ring, created by Australian physicists.

300 nanometres is the thickness of the ring, an elegant plain band of pure synthetic diamond, rather than the traditional stone set in gold.

0 is the number of brides-to-be who will benefit — the nanoscale ring is strictly for studying single photons. But what Sidelines wants to know is: would it still cost a month's salary?

ZOO NEWS

Wild gesture
Kenya's Wildlife Service is giving free entry to under-18s visiting its world-famous national parks until 30 April as a way of "thanking Kenyans for keeping wildlife safe" during the country's recent post-election riots.

Sources: IUCN, Am. Inst. Phys., Environment News Service, Sydney Morning Herald

SIDELINES

Fly's eye detector spies cosmic-ray cut-off

An experiment to detect subatomic particles arriving from deep space has triumphantly announced ... their absence.

The finding, a swansong from the now defunct High Resolution Fly's Eye (HiRes) cosmic-ray observatory in Utah, is far from a disappointment. It is the long-awaited confirmation of a decades-old prediction that there is a critical threshold of energy beyond which these cosmic rays dwindle in number (R. U. Abbasi *et al. Phys. Rev. Lett.* **100**, 101101; 2008). And it adds weight to initial measurements from the Pierre Auger Cosmic Ray Observatory in Argentina.

This energy 'cut-off' was predicted in 1966 by Kenneth Greisen of Cornell University in Ithaca, New York, and in the same year by Soviet physicists Georgiy Zatsepin and Vadim Kuzmin of the Lebedev Institute of Physics in Moscow. They predicted that there would be very few cosmic rays with energies greater than about 6×10^{19} electron-volts (eV) because of energy losses through interactions with the ubiquitous photons of the cosmic microwave background, the radiation that fills the Universe.

But scientists studying high-energy cosmic rays have failed to observe the so-called GZK cut-off. Indeed, ultra-high-energy cosmic rays with energies of up to 3×10^{20} eV have been detected by Earth-based instruments. Such cosmic rays are mainly protons, thought to be generated by awesomely energetic astrophysical phenomena such as supernovae or supermassive black holes.

Most perplexingly, researchers working at a Japanese cosmic-ray observatory called the Akeno Giant Air Shower Array (AGASA) near Tokyo have previously reported a cosmic-ray energy spectrum that shows no obvious sign of a cut-off. "This excited many theorists," says Douglas Bergman of Rutgers University in Piscataway, New Jersey. There was speculation about whether the Japanese results revealed new physics beyond Einstein's theory of special relativity, such as the existence of a 'shortest possible length' analogous to the fastest possible speed (the speed of light) imposed by relativity.

The new HiRes results, reported by Bergman and colleagues, discount such speculation — for now, at least. The team describes a cosmic-ray spectrum that drops sharply at around the predicted GZK cut-off energy. "They've pretty clearly seen the effect," says astrophysicist Alan Watson of the University of Leeds, UK.

One of the main difficulties in spotting the cut-off has been a poverty of statistics. Most cosmic rays have energies lower than the GZK limit, and so it is tricky to detect enough particles at high energies for a drop in the energy spectrum to become clear. Bergman's study drew on almost a decade of data from the HiRes experiment, which ran at the US Army Dugway Proving Ground in Utah from the late1990s until early 2006, when it was shut down.

> "There could be some really exciting particle physics involved here."

HiRes's two telescopes searched the sky for the characteristic flashes of ultraviolet light produced when a cosmic ray collides with a molecule in Earth's atmosphere and creates a shower of secondary particles. The two 'eyes' — hemispheres covered in photomultiplier tubes that look like a fly's compound eyes — capture just about all the light in the shower, giving a good measure of the original particle's energy. "To see the GZK cut-off, it is vitally important to have good energy resolution," Bergman says.

So why hasn't AGASA seen it? "The AGASA people are really good experimentalists, and you can't doubt their measurements," Watson says. But AGASA doesn't measure the cosmic-ray energies directly, so Watson thinks there could be something wrong in the theoretical model used to calculate them — something that might, after all, point to unknown new physics at these high energies. "There could be some really exciting particle physics involved here," he says.

The many photomultiplier tubes of the HiRes detector captured tell-tale signs of cosmic rays.

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Campaign aims to give solar lighting to the developing world.
www.nature.com/news

SELCO

SNAPSHOT
Zooloddities

A sloth is painted in a standing posture by an unidentified artist in the early 1600s. But the arboreal animal, which normally hangs upside down, doesn't have the musculature to support this position. And Swiss artist Maria Sibylla Merian depicts a tarantula carrying a hummingbird, a meal too grand for any real spider.

Many artists had to guess at the typical behaviour of exotic creatures brought to Europe from newly discovered continents. Leonardo da Vinci's anatomically accurate drawings benefited from careful studies of the live animal, as well as dissections. His sketch of a human-like bear foot correctly shows that bears also walk on the soles of their feet.

These and other such gems can be seen at the Amazing Rare Things exhibition, which opened last week at The Queen's Gallery, Buckingham Palace in London.

Anna Petherick

THE ROYAL COLLECTION 2008, HER MAJESTY QUEEN ELIZABETH II

Watson also cautions that the drop in the energy spectrum observed by the HiRes team does not by itself provide conclusive proof of the GZK effect. "It could just be the cosmic-ray sources running out of steam at high energies," he says. However, the new Pierre Auger Observatory, operating while still under construction in the Argentinian pampas, has also seen an apparent dip in the spectrum that is consistent with the GZK cut-off (see *Nature* **448**, 8–9; 2007).

This cut-off is not an absolute limit on the energy of a cosmic ray because the slowing down is cumulative. If a very-high-energy cosmic ray is produced close enough to Earth, it might not lose much energy before it hits the atmosphere. Clearly, this does seem to happen, although astrophysicists are still debating which cosmic events could be energetic enough to create these particles. "The sources of ultra-high-energy cosmic rays we observe must be within about 50 megaparsecs [160 million light years, a typical distance to nearby galaxies] of Earth," says Bergman. There don't seem to be many potential sources that close.

And yet, he says, the high-energy particles seem to come from all directions. This discrepancy remains unexplained. So, although Bergman says the apparent confirmation of the GZK cut-off is "reassuring", he adds that "I would not say all is well with ultra-high-energy cosmic rays". ■

Philip Ball

Stem-cell patents confirmed

An effort to overturn two contested stem-cell patents was quashed last week by the US Patent and Trademark Office, in a move that strengthens the position of the patents' holder, the Wisconsin Alumni Research Foundation (WARF).

The two patents cover methods for deriving and growing primate and human embryonic stem cells (ES cells) in culture. They were challenged in a process called a 're-examination' by a group led by the Public Patent Foundation, based in New York. The challengers claimed that the research carried out by University of Wisconsin stem-cell researcher James Thomson was not novel enough to earn the patents, that the patents were unjustly broad in their scope, and that they were stifling stem-cell research.

On 11 March, WARF released documents in which the patent office affirmed the validity of the two WARF patents, reversing its preliminary decision in March 2007 to overturn them. The decision is final and a considerable victory for WARF.

"If there were doubters out there, they ought to be changing their minds," says Carl Gulbrandsen, WARF's managing director.

Dan Ravicher, executive director of the Public Patent Foundation, plays down the significance of the decision, pointing out that WARF had narrowed some of the claims in its patents. The challengers might try to open a new re-examination process on the modified patents, Ravicher says. "We still think in their modified form [the patents] could be causing public harm. That's why we're going to continue to fight them," he says.

But some say that prolonging the fight might backfire. Ken Taymor, head of the University of California's Berkeley Center for Law, Business and the Economy, says the re-examination has been a boon for WARF, in part because it has diverted attention from WARF's attempts to bolster its patent positions. While the re-examinations were under way, WARF filed actions called 'continuations' that expand the patents' claims, and also filed a series of new patents on cells derived from human ES cells with its commercial licensee Geron of Menlo Park, California.

"The re-examination has strengthened WARF's position," Taymor says. "It has deflected attention from the downstream patent landscape that WARF and Geron have created, and which is much more critical to commercialization than the fundamental patents."

A third WARF stem-cell patent, which had also been challenged, was upheld by the patent office on 29 February. That decision can be appealed, because the rules governing the re-examination of the patent are different. ■

Erika Check Hayden

Revised ozone standard angers environmentalists

The US Environmental Protection Agency (EPA) is once again under fire for ignoring its science advisers — this time in setting a new air-quality standard for ozone, a primary component of smog.

The agency's decision on 13 March will reduce the current regulatory limit on ground-level ozone concentrations, set in 1997, from a maximum of 84 parts per billion (p.p.b.) to 75 p.p.b.. An agency advisory panel had recommended a range of 60 to 70 p.p.b..

Environmentalists also criticized what they saw as White House interference. In a 6 March memorandum, a top White House regulatory official, Susan Dudley, urged the EPA not to set a secondary "welfare" standard at a lower level to protect against other environmental problems, including potential damage to crops and other vegetation. In the end, the EPA set the primary and secondary standards at the same level.

The air quality in an estimated 345 municipalities and counties could be in violation when the new standards come into effect in 2010.

Fresh safety concerns for popular anaemia drugs

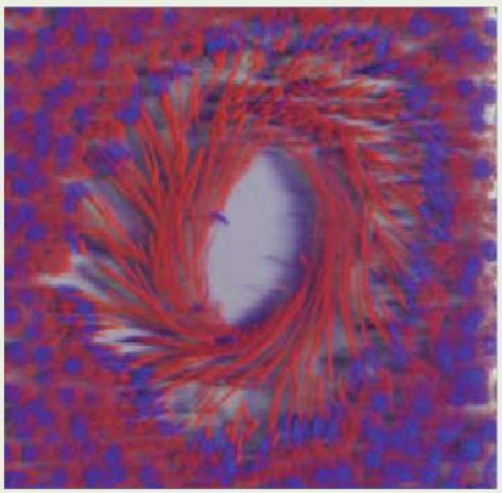
A panel of advisers to the US Food and Drug Administration (FDA) last week recommended restricting the use of several blockbuster anaemia drugs in cancer patients.

The drugs, which promote the production of red blood cells, are Aranesp and Epogen, sold by Amgen, and Procrit, sold by Johnson & Johnson. These medicines are the subject of safety concerns after recent trials linked them to shortened cancer survival times and increased tumour growth.

The advisers voted overwhelmingly to keep the drugs on the market for treatment of anaemia induced by chemotherapy, but said that they should be used only with specific cancers. They voted by a majority of nine to five to bar the drugs' use in patients with head and neck, and breast cancers, for which studies had raised particular safety concerns.

The FDA is not bound to implement its advisers' recommendations, but it often does.

Wellcome awards reveal microscopic masterpieces



MRC NIMR

This picture of sperm developing in a testis is one of 23 winning images in the 2008 Wellcome Image Awards, which focus on the visual wonder of the microscopic world.

The cell nuclei are shown in blue and the mitochondria appear red in this striking image taken by Kate Sullivan, an electron microscopist at the Medical Research Council's National Institute for Medical Research in London. The tails of the sperm are pointing inwards.

Other images from the competition, including a fly standing on sugar crystals and an array of vitamin C crystals, can be found at www.wellcome.ac.uk/en/wia/gallery.html.

NASA/JPL/SPACE SCIENCE INSTITUTE

This image of Enceladus was captured last week.

Saturn flyby is a success despite computer glitch

NASA's Cassini spacecraft swooped past Enceladus, a tiny moon of Saturn, as planned on 12 March. But the dust analyser onboard failed just as the probe flew through the ice and gas plume that spews from the moon's south pole (see *Nature* 452, 139; 2008).

The glitch arose two hours before the flyby, when the team responsible for the instrument tried to upload a software patch to speed up its counting rate to as high as 100 particles per second.

Uploading the patch took only a few microseconds, but unfortunately coincided with a higher-priority command from the main spacecraft. The patch fizzled. "It's really frustrating," says Sascha Kempf, deputy principal investigator for the instrument at the Max Planck Institute for Nuclear Physics in Heidelberg, Germany. Kempf says the team can update the software in time for the next flyby in August.

The 11 other data recorders on the spacecraft worked fine. "We're disappointed in the one instrument," says Dennis Matson, project scientist for the mission at the Jet Propulsion Laboratory in Pasadena, California. "But it's more than outweighed by all the great stuff we did get."

Bonn to play host to dementia research centre

The German science ministry is providing €40 million (US$ 62 million) to set up a research centre in Bonn to study neurodegenerative conditions such as Alzheimer's disease. A further €20 million will be distributed to six partner organizations in locations such as Munich and Tübingen.

Some 300 staff are expected to work at the German Centre for Neurodegenerative Diseases. A detailed research programme will be agreed in the next few months.

The new centre will come under the umbrella of the Helmholtz Association, which runs 15 national research institutes. It will be the first new Helmholtz centre to be founded since the country's reunification in 1990.

Korean institute inquiry prompts two retractions

A team led by a senior South Korean scientist is retracting two papers, in *Science* and *Nature Chemical Biology*, following a university investigation into the research.

Tae Kook Kim, of the Korea Advanced Institute of Science and Technology (KAIST) in Daejeon, had reported a new method for imaging living cells that used magnetized nanoparticles (J. Won *et al. Science* 309, 121–125; 2005, and J. Won *et al. Nature Chem. Biol.* 2, 369–374; 2006). KAIST's investigation is ongoing, but last month it reported preliminary findings that the "scientific truth" of the papers was in question. Investigators are also probing whether misconduct is involved.

Terry Sheppard, editor of *Nature Chemical Biology*, says that the KAIST investigation team told him that the authors wanted to retract the paper. The retraction, he notes, will occur "as soon as possible". *Science* also confirms that the team is working to retract its paper.

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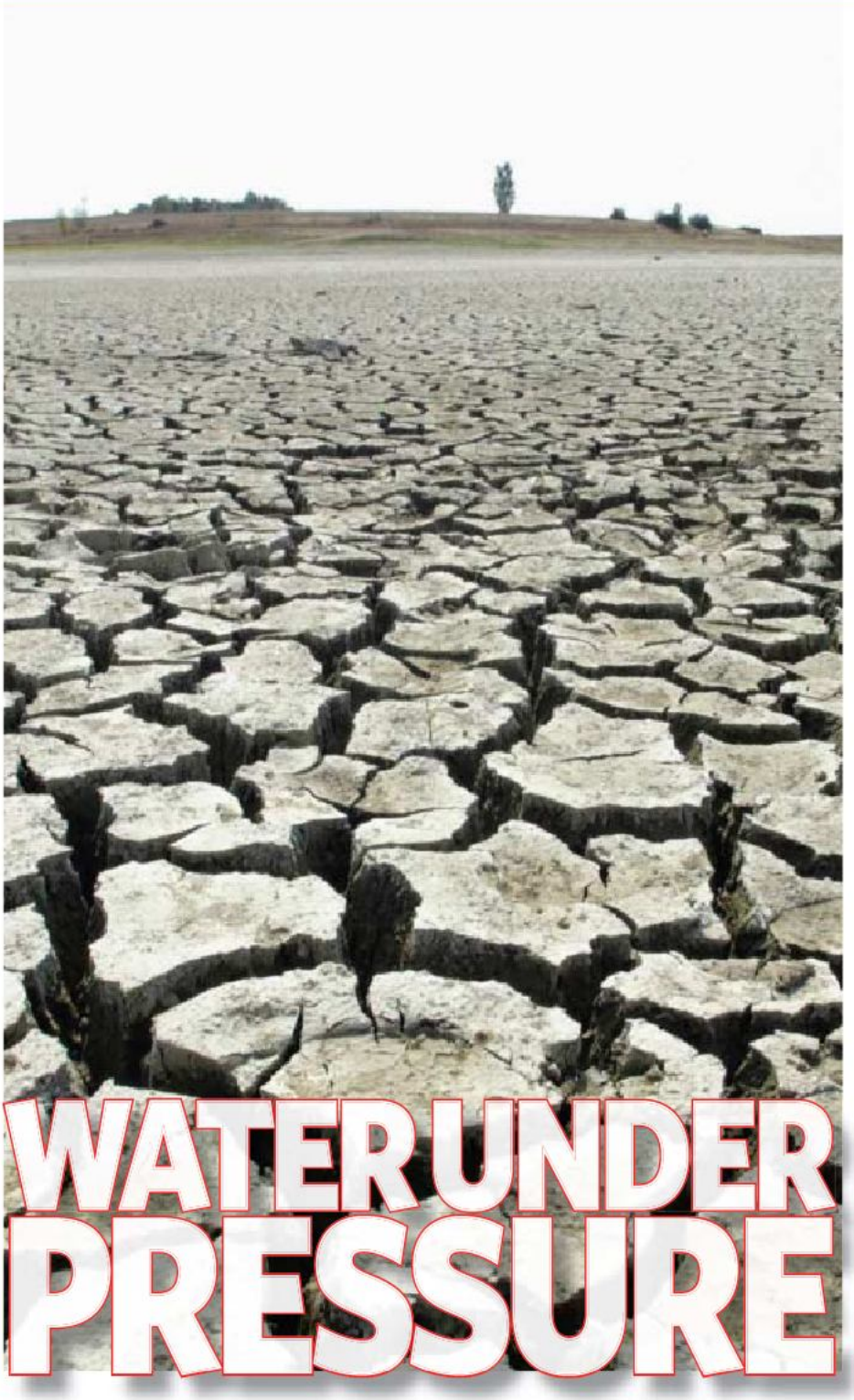


WATER UNDER PRESSURE

L.BONAVENTURE/AFP/GETTY IMAGES

More than a billion people do not have access to safe drinking water and two billion have inadequate sanitation. This is despite two international decades, a millennium declaration goal, two international years and a string of global celebratory days — all dedicated to drinking-water or sanitation. Why has progress been so slow?

One reason could be that the current global targets — the Millennium Development Goals — do not provide sufficient incentives for all nations to ensure that everyone has access to water and sanitation (see page 283). Another reason is that the pressures on water resources are continuing to rise: whether through population growth, economic development or climate change (see page 270).

The effects of water shortages are already spilling over from health and sanitation into key economic activities such as agriculture and energy production. Here the challenges are clear, if not fully appreciated: agriculture could easily require twice as much water in the next few decades (see page 273). And the global demand for energy is projected to rise by 57% by 2030 (see page 285).

It we want to improve global access, it is time to rethink our strategies to water, and to respond to global trends in food and energy production.

New concepts are emerging, with experts and policy-makers stressing simple solutions to improve crop yields in the rain-fed areas where many of the world's rural poor live. This is preferable to expanding the area of irrigated farmland, which is already the biggest consumer of freshwater worldwide.

Growing energy demands will require a more integrated strategy for managing freshwater — to prevent cities and farms, or upstream and downstream users, from coming into conflict. This sort of joined-up policy-making has been sorely lacking, but will be crucial to water management at the river basin, and at both regional and transnational scales (see page 253).

Elsewhere in the issue, a Review Article (see page 301) highlights the purification technologies that scientists hope will improve access to safe drinking water. An Essay (see page 291) explains how physicists still argue over theories about the structure of water. And Books & Arts reviews a television documentary on the privatization of water supplies (see page 288). ■

A LONG DRY SUMMER

In parts of the world already facing unreliable food supplies, an uncertain climate adds to the future stress for soils, plants and people. **Quirin Schiermeier** reports on water strategies for a drier world.

The record-breaking European heatwave of 2003 did not come out of the blue. It was preceded by an unusually dry spring during which soils dried up across the continent. The lack of moisture resulted in strongly reduced soil evaporation and cooling, which in turn intensified the temperature extremes during the summer.

Climate scientists believe that in the second half of this century, extreme summer heat and drought could become the rule rather than the exception as global temperatures rise. In any case, rapid loss of soil moisture early in the year now seems to be a signal for subsequent summer heatwaves in Europe[1]. A feedback loop appears to be at work: as heat dries up the soil, the dry soil amplifies the heat.

Changes in soil moisture content may have other feedbacks, affecting soil erosion, surface runoff, soil nutrients and even cloud formation. But predictions of soil drying in response to rising temperatures are still very uncertain. For Africa and South America, climate modellers are not even confident about the sign of the simulated changes.

"We are told climate variability will increase and that it may get drier in some regions, but we really know too little about the details," says Malin Falkenmark, a hydrologist and water-management expert at the Stockholm International Water Institute in Sweden. This uncertainty hasn't stopped Falkenmark, along with other hydrologists, from recommending changes to water-management practices in response to climate change, and to declare an end to the wait-and-see approach of the past[2].

"We don't know for sure how climate change will unfold, but there's no doubt any more that it is happening and that there needs to be some preparedness," Falkenmark says. "River flow in some dry regions may decrease by up to 40%, for example. That must alter water-resource planning methods. We cannot just wait until it happens."

Current models suggest that more rain will fall, but less often, leading to longer periods during which soil moisture is critically depleted. Observations from several regions, including North America, Europe, southern Africa and Australia, confirm a trend towards heavier rainfall events, with longer dry periods in between, particularly during the summer[3].

Down to earth

Observable trends for soil moisture are more elusive. As yet, soils seem to be more resilient to global warming than, say, mountain glaciers or polar ice sheets. In the few regions where good records are available — such as the Ukraine, where scientists have measured soil moisture for 45 years — researchers have found no evidence for much of a downward trend, if any.

"Soil moisture is not an easily measured quantity," says Jerry Meehl, a climate researcher at the US National Center for Atmospheric Research in Boulder, Colorado, and a lead author for the Intergovernmental Panel on Climate Change (IPCC). "The IPCC first predicted increased mid-continental summer drying of soils almost 20 years ago," he notes. In the absence of observations to support or refute this prediction, the science has not advanced much since then.

Climate models are consistent in predicting greater summer soil dryness after 2050 in parts of every continent except Antarctica. But where that will change, and how much, depends heavily on the model (see maps, below), none of which are yet good enough to allow detailed soil moisture predictions at the river-basin scale or below — the scale that matters to water-management experts such as Falkenmark.

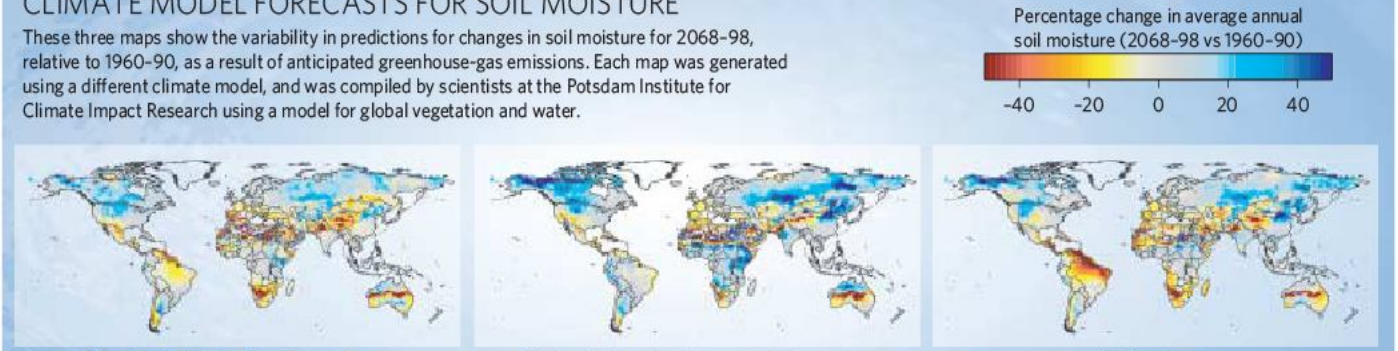
The main reason for the fuzziness is that it is much more difficult to model rainfall than temperature. The processes that control rainfall, such as cloud and droplet formation, occur on much smaller scales than are used by existing climate models. Soils are also too patchy to be reliably represented in current models. Finally, the complex interactions between rainfall, evaporation, carbon dioxide concentration, plant growth and soil moisture are not easily computerized.

Because soil moisture and rainfall influence each other, the models desperately need better soil data to improve. Yet the world's soils are not nearly as well monitored as temperature or precipitation; *in situ* observations are few and scattered. To disentangle the complex interplay, scientists would need to find some way to measure soil moisture content directly and continuously.

There is hope that satellite measurements will help. Both the European Space Agency (ESA) and NASA are planning missions to

CLIMATE MODEL FORECASTS FOR SOIL MOISTURE

These three maps show the variability in predictions for changes in soil moisture for 2068–98, relative to 1960–90, as a result of anticipated greenhouse-gas emissions. Each map was generated using a different climate model, and was compiled by scientists at the Potsdam Institute for Climate Impact Research using a model for global vegetation and water.



J. P. ARLES/REUTERS

Feeling the heat: the frequency of droughts like that seen in Europe in 2003 is likely to increase.

observe soil moisture, expanding the work of ESA satellites ERS-1 and ERS-2. The microwave sensors on board the planned missions will give almost global coverage of soil moisture changes in real time. Where dense vegetation hides the soil, greenness can be used as a substitute.

At the same time, increasing computer power is allowing researchers to improve their models. There's still some way to go, admits Peter Cox, a climate modeller at the University of Exeter, UK. Current models are not yet fine-grained enough to model individual tropical storms, for example. But Cox says that some regional models are getting close.

"The trick is to use various sources of information and fuse them together so as to construct a global data set," says Cox. "As climate models and satellite observations are converging in scale and resolution, we can start ingesting satellite data into our models and make them more powerful."

> **"In Africa, 'rainy season' means that rain can fall, not that it will fall."**
> — Malin Falkenmark

The public usually associates water shortages with a lack of drinking water. But global water scarcity is primarily an issue of hunger, not thirst. Declining soil moisture generally means an increasing risk of drought. Monitoring and understanding possible soil moisture changes is therefore vital for crop management in all regions at risk of water scarcity.

Researchers expect the most severe impacts to occur in the transition zones between wet and dry climates. In very wet regions, where soil water is always plentiful, evaporation and precipitation are hardly sensitive to soil moisture. And in very dry regions the rate of evaporation is too small to generate much precipitation anyway.

In one of the best available estimates, a multi-model study conducted by the Global Land–Atmosphere Coupling Experiment, run by the World Climate Research Programme, the hot spots of coupling between soil moisture and precipitation appear in the plains of North America, sub-Saharan Africa and northern India[4]. These regions, and in particular the 'hunger belt' from the Sahel to the Horn of Africa, are thought to be most at risk from the effects of climate change, such as more frequent droughts and floods, and accelerated soil erosion.

Soils store rainfall in the root zone of plants. This is called 'green water', as opposed to the blue water in rivers, lakes and groundwater stores. In dry regions, blue water is usually very scarce, often accounting for less than 10% of the overall water balance. All rain-fed agriculture in tropical and savannah regions, where irrigation is minor, depends on soils' capacity to capture what little rain falls.

"Green water is the key to water and food security in drought-prone regions," says Falkenmark, who coined the term in the early 1990s. But experts believe that only 10–30% of rainfall in the world's savannah belt — the dry to moderately wet zones on all continents — is being used in a productive way.

The effect of climate change on water scarcity in regions that lack food security is becoming evident. Given the degree of human interference with climate and water, Falkenmark and other international experts recently declared dead the idea that water planners need consider only natural variability (and not human influence) when managing water supplies[2]. What the developing world needs now is a second 'green revolution', aimed at increasing yields by improving green-water management, soil conservation efforts, and more efficient protection of crops from prolonged dry spells, she says.

Green and blue water are not separate resources, of course. Irrigation turns blue water into green (see graphic, overleaf). But in dry regions it is difficult to improve water availability through engineering works such as dams. "It is very unsatisfactory, therefore, that

Keep it simple

The year 2000 was another dry one in Kenya. With little rain during most of the growing season, the president declared a state of emergency and called for international food aid to support the starving population.

But while crops failed across the country, some corn (maize) growers in the Machakos district southeast of Nairobi had bumper yields of up to 3 tonnes per hectare.

This was not luck. The farmers had built a few small dams upstream, and stored some water from a downpour early in the season. It was only enough to fill two small swimming pools, but it was enough to bridge the dry spell.

What the Kenyan government called a severe meteorological drought, was really just an agricultural drought, says Johan Rockström, a water-management expert at Stockholm University, Sweden. There was plenty of rain, but most of it fell in one downpour at the start of the rainy season.

Rockström and his team are helping farmers in Kenya, Ethiopia, Uganda and Tanzania make better use of soil and erratic rainfall. Collecting water from local runoff, for irrigation during dry spells, is one plan. They are also advising farmers to switch from ploughing to tilling.

In the past 20 years, tilling has transformed farming in Latin America. By lightly tilling the soil rather than turning it with a heavy plough, farmers avoid forming hard troughs that hinder rainwater from entering the soil and can increase soil erosion. And if soils are not turned, less of the organic material that holds water will be exposed and get lost to the air by oxidation. A welcome side effect, climate-wise, is that more carbon stays in the ground.

But many African farmers hesitate to abandon the plough and return to tilling. Ploughing, which was introduced during colonial times, keeps weeds down. Poor farmers also worry about investing in new technology and expensive fertilizers for fear of losing their investment through crop failure.

Experts believe that maximizing rainfall infiltration into the soil, alongside water harvesting for irrigation, is key to producing more food in rainfed agricultures. If it gained wider acceptance, tillage alone could vastly improve water availability in savannah regions, says Rockström.

Simple means of improving water storage in agricultural soils could quadruple yields of important crops, says Rockström. "Climate change doesn't make the task easier," he says. "But we're certainly not hopeless." **Q.S.**

most water engineers are still mainly thinking in blue-water terms," says Falkenmark.

To capture green water in dry African regions, farmers need to make sure that enough rain can infiltrate the soil after dry spells, for example by adopting more soil-friendly ploughing techniques, which have already increased yields in Latin America. And experts recommend that farmers harvest water from local runoff to use during dry spells in the growing season (see 'Keep it simple').

Going green

Even without climate change, rain in the savannah belt is erratic. In sub-Saharan Africa, for example, dry spells typically occur even in 'wet' years. "In Africa," says Falkenmark, "the term 'rainy season' means that rain can fall, not that it will fall."

For soil moisture and green water, the local frequency and intensity of rainfall are at least as important as the total amount of precipitation. Heavy rain cannot penetrate parched and crusted soils, and without efficient water and land-use management, researchers warn that more variable rainfall in vulnerable regions threatens to increase runoff, erosion, water stress on plants and flooding.

> "Global water scarcity is primarily an issue of hunger, not thirst."

Models agree that global warming will amplify the entire hydrological cycle, from evaporation to precipitation to runoff[5]. Global precipitation over land may slightly increase, especially in some northern latitudes or tropical regions, with a greater fraction occurring during the heaviest events.

Markus Reichstein, a carbon-cycle expert at the Max Planck Institute for Biogeochemistry in Jena, Germany, has studied the consequences of more extreme rainfall on ecosystems. He says all levels and processes of the ecosystem are likely to be affected, from runoff to soil evaporation and nutrient availability. Changes will affect all climate zones, but some ecosystems may respond very differently to others, a 15-strong interdisciplinary team concludes in its as yet unpublished review.

This irrigation system in Tanzania captures runoff water to boost water supplies in dry periods.

R. KAUTSKY/AZOTEIMAGES

Plants' ability to adapt to changing water and nutrient availability might be crucial for their survival in a warming world. Ecologists think there are thresholds beyond which plants become stressed. But these vary between ecosystems, and so may plants' responses to climate change.

Soil water availability generally limits plant growth and photosynthesis. But nutrient availability in soils increases during dry spells, which suppress nutrient uptake by plants more severely than nutrient mineralization.

Still, in all semi-arid regions more extreme rainfall will increase stress on crops and vegetation, scientists believe[6]. Unfortunately, these are also densely populated regions with unreliable food production. In sub-Saharan Africa, longer dry spells will harm vegetation and, without supplementary irrigation, decrease yields.

A question of breeding

How best to adapt? The 2003 heatwave, which reduced yields in some European countries by more than 50%, shows that the rich world is not immune from the consequences of a warming climate, and from the need to adapt. But climate change is without doubt a much bigger threat to food security in poorer regions.

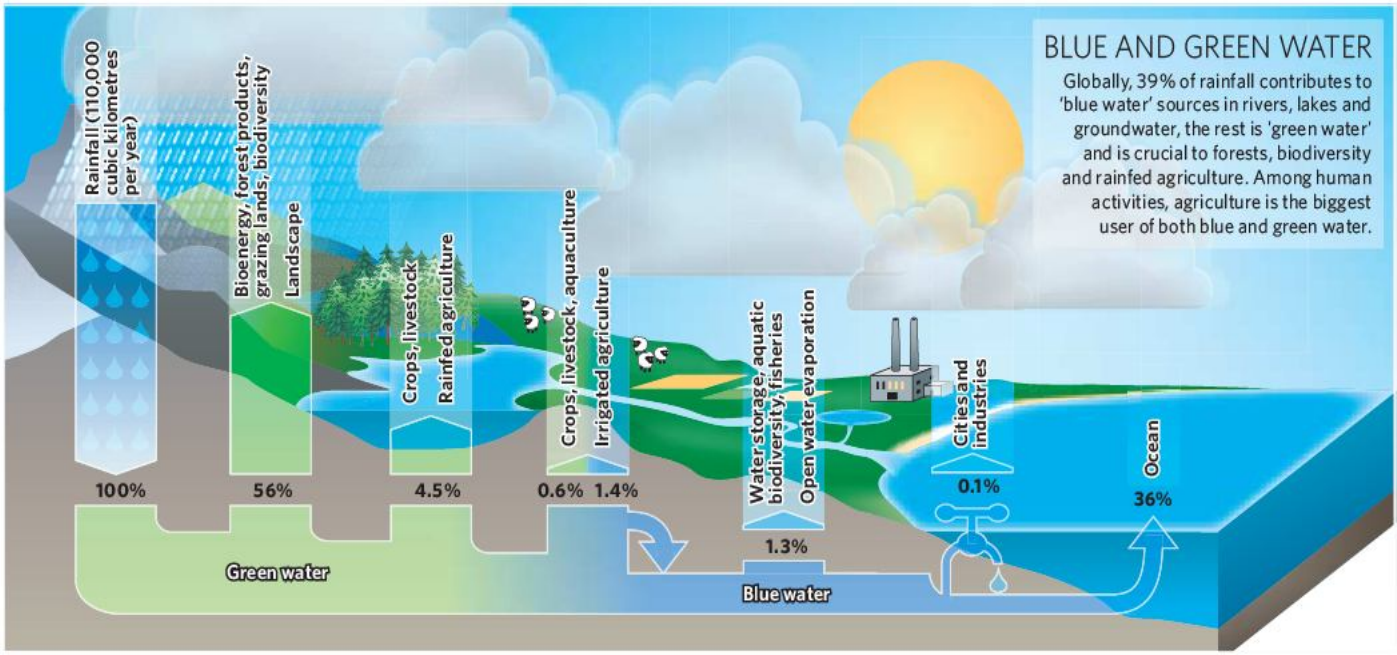
Experts warn that poverty tends to entrench the deficiencies of rain-fed agricultures in developing countries. As poor farmers cannot afford to invest in their crops, foreign investment aid or cheap loans are vital.

A recent analysis of climate risks for crops in 12 regions with food insecurity shows that crops such as oilseed rape, corn (maize) and wheat in south Asia and southern Africa are most vulnerable. Agricultural investment and adaptation efforts should focus on these crops and regions, the authors suggest[7].

"We're seeing a massive challenge," says David Lobell, an agricultural ecologist at Stanford University in California and one of the authors of the study, who warns that plant breeding is under-resourced.

"We must urgently develop new crop varieties tolerant to heat and drought, and not just maize," he says. "And we need to work hard and very quickly on it. Don't forget it can take 15 years of development effort until a new variety is adopted by farmers."

But without prior investment in water and land management, crop-adaptation efforts will be less effective, says Deborah Bossio, director of research at the International Water Management Institute in Colombo, Sri Lanka, and a lead author for the International Assessment of



Agricultural Science and Technology Development, an international effort akin to the IPCC for agriculture.

Investment in water is particularly essential in south Asia and sub-Saharan Africa, says Bossio. And it should consider the full range of water storage and delivery options, she says, from the most local — soil water storage and farm ponds — to community projects such as small reservoirs.

But she warns that too much focus on crop production may put crops and livestock into conflict over water, with the risk that vulnerability is increased. "Livestock are always a very important component of the livelihood systems in areas at risk from water scarcity," says Bossio. Adaptation to water scarcity has to consider all the components that affect people's lives. ■

Quirin Schiermeier is a reporter for *Nature* based in Munich.

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See Editorial, page 253.
For more on water see www.nature.com/news/specials/water/index.html.

MORE CROP PER DROP

Farmers' yields in the developing world are often limited by unreliable rains. Improving their harvests will require plant breeders, agronomists and geneticists to pull together — but can these experts work out their differences? **Emma Marris** reports.

The International Assessment of Agricultural Science and Technology was to be to agriculture what the Intergovernmental Panel on Climate Change is to climate: the definitive statement of the scientific art. Hundreds of researchers have worked on the report for five years. It is co-sponsored by the United Nations, the Global Environmental Facility, the World Bank and the World Health Organization, and included in its vast pool of stakeholders are big companies, small farmers and scientists from around the world. But this January, CropLife International, the trade group that represents crop-science giants including Monsanto, DuPont and Syngenta, walked out.

At issue was the report's handling of the role of biotechnology in the developing world — or rather, the degree to which it chose to ignore that role. The crop-science companies think complex genetic traits will be a crucial part of the future of developing-world agriculture; the draft report, though, suggests that genetically modified (GM) crops have little to offer in this regard.

Because water (either from the sky or the irrigation canal) is often a key factor in determining crop yields, squeezing more crop out of the same drop (see 'Virtual water', page 275) will be central to one of the biggest challenges of this century: sustainably feeding a population of perhaps 9 billion people in a climate-changed world where rain, temperature and drought will be increasingly erratic. Already, 1.2 billion people live in areas where there is not enough water for everyone's needs[1] (see map, page 275), and that figure will probably grow faster than the overall population of the planet. Everyone agrees on the problem, but as the CropLife walkout demonstrated, not everyone agrees on the solution.

"Resources for GM development have been

J. PIETRAGALLA/CIMMYT

spread very abundantly, with a great deal of overselling," cautions Pasquale Steduto, Italy-based chief of the United Nations Food and Agriculture Organization's Water, Development and Management Unit. "So far, we do not have a direct gain from GM or molecular biology in terms of drought resistance."

For Steduto, raising the maximum yield for a given crop with a given amount of water is not as useful as getting the many millions of low-yielding small farms up to where the efficient Western farms are now. For example, wheat, he says, seems to have an upper yield boundary of about 22 kilograms per hectare per millimetre of water per year. "The upper boundary is like an envelope, in which you see all sorts of productivity from almost zero to very close to this limit," he says. Agronomic techniques can be used to fill the envelope worldwide.

Yielding to technology

A recent report from the International Water Management Institute, one of the groups within the Consultative Group on International Agricultural Research, makes a similar point this way: "Seventy-five per cent of the additional food we need over the next decades could be met by bringing the production levels of the world's low-yield farmers up to 80% of what high-yield farmers get from comparable land." That is, land with similar soil and rainfall patterns. For example, says David Morden, the report's lead author, "grain yields in Uganda are on the order of 1 to 2 tonnes per hectare, and in a similar environment, one could expect 6 to 8 tonnes with really good management."

Much of the difference, the report says, can be made up by disseminating basic research techniques such as choosing a wise mix of crops and livestock for each plot or creating small dams or terraces for water management. Often, real gains are within an individual farmer's reach economically, if only they knew what they were. In other cases, increasing yields requires modest investments in technologies or inputs that could be provided by micro-investment or donations. And finally, better management at the country and international level will make for stronger markets and more reliable water supply (see 'Wilting watersheds', page 277).

> "Rocks and stones are drought tolerant, but plants need water. It is quite limited how much you can tune that."
> — Matthew Reynolds

Many in the world of agronomy and rural development see little role for biotechnology in these efforts. Despite vigorous adoption rates in the developed world — 60% of biotech crops are grown in developed countries[2] — it is conventional wisdom that biotechnology is at best a mixed blessing for the developing world. Some of this dismissal could reflect scepticism about the motives of seed companies. But those who work with genes say that they view it as impatience and a lack of faith in technology that is taking time to mature.

That's because the first GM crops were simple and came easy, says Marc van Montagu, a researcher at Ghent University in Belgium. He revolutionized agricultural biotechnology with the *Agrobacterium* method of introducing new genes into plants. But the game has moved on, and traits far more complicated than pesticide resistance are tougher to crack. "Drought tolerance looks dramatically complicated, but it can be done," van Montagu says. And it can be done only with biotechnology, he insists, "The best of traditional breeding is too slow."

Roger Beachy, head of the non-profit Donald Danforth Plant Science Center in St Louis, Missouri (partly funded by biotech giant Monsanto, across the street), admits that scientists promised too much too early in the field of drought tolerance. "There were some remarkable and extravagant predictions back in the 1980s," he says. "It comes back to haunt you." He compares the early buzz on genes associated with drought tolerance to the discovery of the first gene associated with breast cancer — *BRCA1*. People celebrated an imminent end to the disease, "and then they found a second and a third and a fourth and a fifth gene, and they realized it was more complicated".

This impatience is certainly felt by traditional plant breeders such as Marianne Bänziger at the Mexico-based International Maize and Wheat Improvement Center (CIMMYT). "At the moment there are probably hundreds of groups that work on transgenic drought tolerance," she says, "but very few have made it into the field and shown yield increases." She's also worried that transgenic approaches steal limelight — and funding — from traditional plant breeding, which is itself becoming much more powerful with the help of new genetic techniques that can speed up field-based breeding. Marker-assisted selection, for example, allows plant breeders to follow genetic markers linked to specific genes of interest.

In the long run, biotech researchers say, impatience is counterproductive. Beachy thinks the backlash against biotechnology is a grave mistake. "We are still in the infancy of advanced agriculture," he says, "and in the infant stages everyone thinks that their piece is more important than the next. As the world begins to recognize the severity of the problem, we will all become more collegial — we need everyone on board with all the tools in the arsenal."

Plants need water in all sorts of ways; without

A wheat researcher in Mexico measures crop temperature using an infrared sensor to estimate root depth during drought stress.

Virtual water

The goal of 'more crop per drop' can be tackled on several levels — the individual plant, the whole field, the whole river basin or the whole Earth. But efficiency gains on one level don't always improve the situation on the other levels. An arid country may find that it is more cost effective to give up producing commodities that require lots of water and buy them from abroad.

For example, a kilogram of modern industrial beef, according to Arjen Hoekstra, a water-management specialist at the University of Twente in the Netherlands, takes 15,500 litres of water to produce. So, rather than import all that water, why not instead import beef and concentrate your energies on an industry better suited to a dry country?

So far, just a few countries have taken the 'virtual water' idea on and folded it into their policies. Jordan stands out, consciously trying to increase its imports of water-intensive food. Policies such as these go against the grain in many countries, where self-sufficiency is often prized. But, as one might expect, some countries, such as China, that have increasingly felt the pinch of water scarcity have been "unconsciously" turning to virtual water imports[6].

Virtual-water researchers think big, in cubic kilometres of water. So do the possible genetic gains at the plant level even make it into their equations? "I see it as one of the many ways to try to reduce water needs," says Hoekstra. "Sometimes, in some cases, it can be a partial solution." **E.M.**

it they can't absorb nutrients or photosynthesize — and water pressure keeps green plants from wilting. To think plants can be made drought-proof is a mistake, says Matthew Reynolds, a wheat physiologist at CIMMYT. "Rocks and stones are drought tolerant, but plants need water. It is quite limited how much you can tune that."

What's more, the ways that plants deal with water stress when left to their own evolutionary devices may not suit the needs of farmers. One example is seed abortion in corn (maize). "Under drought conditions," says Bänziger, CIMMYT's director for corn research, "the maize plant puts more resources into pollen formation and less into seeds." From the plant's point of view this makes sense. Pollen is much cheaper energy-wise for the plant to make, and, if the pollen manages to fertilize another plant's seed, the drought-afflicted parent will still contribute 50% of its genes to the offspring. But this is of little help to farmers, who sell kernels, not pollen.

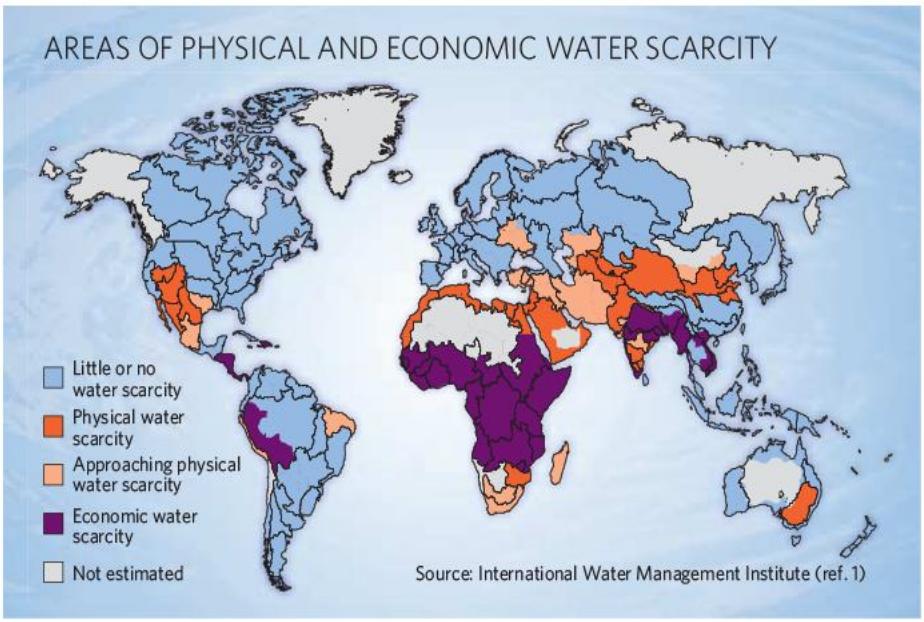
So one option is to stop the plants from doing what comes naturally during drought. "Plants normally avoid stress," says Eduardo Blumwald, a plant biologist at the University of California, Davis. "Actually we all avoid stress. If it is very dry, we go to the pub and have a beer." Tobacco plants close their stomata — the pores through which water is lost — and start shedding older leaves to reduce the area that transpires. "That strategy has worked for millions of years," says Blumwald, "it is a good strategy."

On a farm, though, it means that a lot of proteins and sugars that could end up in seeds are shed. So Blumwald engineered tobacco plants to retain a plant hormone called cytokinin in older leaves, so although they drooped, they weren't shed. When these modified plants are rewatered, the old leaves spring back, and the goodies inside them are available for seed growth[3]. "We find no cost; there is no yield penalty," says Blumwald, who says that one of the most common side effects of tweaking genes is a drop in yield.

Blumwald's work is still in model plants such as tobacco, however. Bänziger notes that a lot of transgenic research is done on another model plant, thale cress (*Arabidopsis thaliana*). She also worries that getting from lab to farm is hard and under-resourced. Even when research is done in field crops, she says, "it never gets past the lab bench because the environment in the field is very different".

And it is with traditional selective breeding, not GM techniques, that Reynolds and colleagues have developed several promising varieties of wheat that have higher yields in drought conditions, some of which are within a year or two of distribution. Some are crosses between modern lines and wild relatives of wheat from the Middle East, where the crop originated. These lines change their root architecture in drought conditions, going deeper.

Others take advantage of an accidental 500-year breeding experiment. Wheat came to Mexico with the conquistadors so that they could make bread for Catholic mass, Reynolds says, as "maize was considered a pagan crop". In the half-millennium since, farmers have adapted it to the dry local conditions. Many of

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"Resources for GM were spread very abundantly, with a great deal of overselling."
— Pasquale Steduto

these strains have very deep roots[4].

Often the same targets lend themselves to both breeding and biotech approaches. Some research avenues involve closing down stomata, others aim to pump up the water conductance of the whole plant or adjust leaf architecture to maximize photosynthesis even in wilting heat. Various techniques to make plants use carbon dioxide more efficiently — and thus keep their stomata more tightly shut — are under discussion. Such carbon dioxide concentration mechanisms have evolved naturally in various plant families.

Like many large seed companies, Pioneer Hi-Bred based in Johnston, Iowa, a business owned by chemicals giant DuPont, is working on both transgenic and traditional ways to extend the photosynthesis of a plant under drought stress, ways to dodge the dreaded seed abortion and ways to jiggle the plant's schedule around the calendar to keep its vulnerable flowering season out of the hottest weeks.

All these approaches begin with what is known about how plants deal with drought stress, and traditional breeding can't really jump off from anywhere else. But there are those who take a purely genetic approach to tweaking a plant's relationship with water. This includes mining plant genomes for any genes that seem to have something to do with drought response, and then tinkering with them. "It is really about characterizing the function of genes, genome-wide," says Jacqueline Heard, Monsanto's project leader for drought-tolerant corn.

Using a brute-force approach, Monsanto and Mendel Biotechnology of Hayward, California, are systematically investigating the transcription factors in the crops they work with, knocking them out or over-expressing them to see what they get. Transcription factors are genes that turn other genes on or off, and so they tend to be higher up in the cascade of changes that might unfold when a plant experiences stress. Monsanto and Mendel have found some promising contenders in model plants[5], and Monsanto has two drought-tolerant corn products in development, for delivery around 2015, that sprang from this approach.

"Our first products were all about weeds and bugs; we really believe that the next decade is going to be about yield," says Steve Padgette, Monsanto's vice-president for biotechnology research. He adds that although drought tolerance is indubitably more complex than the traits the industry has worked with before, research is catching up with the complexity. "The science is more tractable and the market is pulling," he says. William Niebur, vice-president for Crop Genetics Research and Development at Pioneer Hi-Bred, says that the company sees a market for drought-tolerant crops across all regions and at all scales, but the products, and the profits, may be long in coming. "This is much more complex than identifying a protein that will kill an insect or make a plant withstand a herbicide," says Niebur. "We see this as an area where we will spend our entire careers and there will still be room for improvement."

Down on the farm

Thanks to the constellations of funders and companies, it is nearly impossible to get global statistics that would show whether transgenics or agronomy is getting more money.

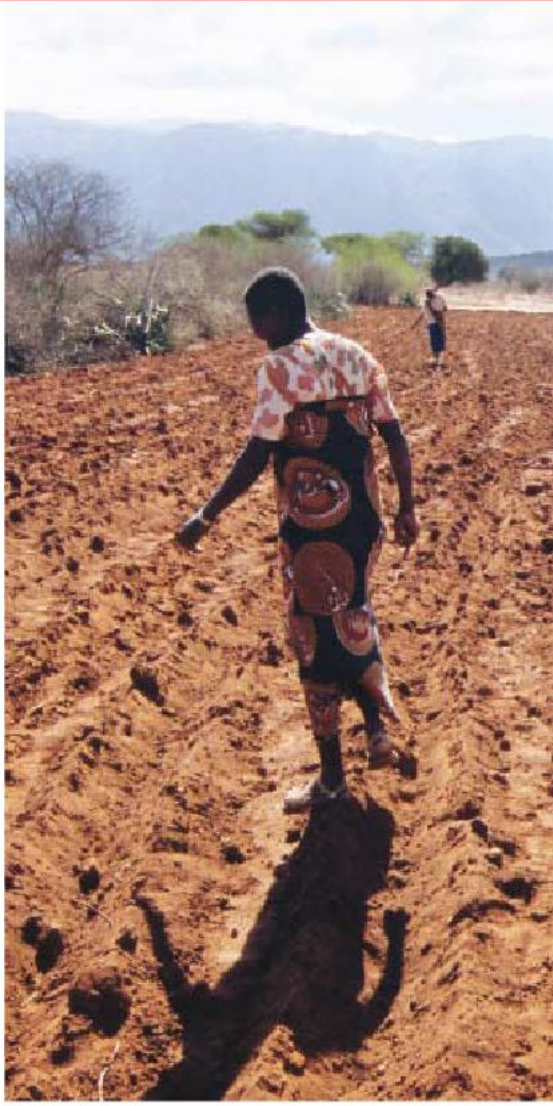
"I would guess that more money is put into genetic manipulation," says Reynolds, because "it is a lot easier to get a return on your investment. It is hard to patent an agronomic manipulation." And this, he thinks, is "positively dangerous. If we don't take care of the soil with the right agronomic strategies, then all our genetic manipulation will be futile."

This is why the world needs farm-level agronomists, detail-oriented and muddy-booted. Among them is Hubert Savenije, a hydrologist at the Delft University of Technology who has helped some Tanzanian farmers manage their farms better. "The biggest gains we can make are clearly when the rain infiltrates the soil," he says.

In his study areas in Tanzania, farmers practise a kind of specialized terracing called *fanya juu*, in which a series of trenches are dug perpendicular to the land's slope with soil heaped on their upslope sides. As water runs down, the heaps catch water for the plants immediately behind them. After several years of cultivation, such land looks like a series of lipped steps. Another technique is to cut the soil with a knife and plant corn seed in a 50-centimetre-deep slit, so that it roots deeply. And some farmers gather small amounts of water draining off fallow land, or a road. Increasing root depth, reducing evaporation of water from the soil and scavenging water to add back to soil are not new concepts to developing-world agronomy. But doing it right is difficult. "Every case is very specific and you have to experiment from a lot of options," says Savenije.


OREGON STATE UNIV.

"We need everyone on board with all the tools in the arsenal."
— Roger Beachy



Iddi Murindaka, a farmer Savenije works with near Mwembe in Tanzania, uses *fanya juu*, spreads manure and diverts some water from a nearby gulley onto his crops. He remembers the last bad drought well. "We had no food; our livestock died; my family was in very poor health," he recalls. "We would sell two goats to buy 20 kilograms of maize." Now things have improved; his household boasts a recently purchased sewing machine and his children are able to attend school.

He and the other farmers Savenjie works with are interested in crop varieties that would mature early, beating the droughts, and thus make the most of their new farming practices. When asked which approach was more important, they all say "both". "Even with proper farm management, a poor seed will take longer," says Murindaka.

R. KAUTSKY/AZOTE IMAGES

Tanzanian farmers want crop varieties that mature early to beat the drought as well as good agronomics.

But that does not mean he would invest in a better seed, even if available, because the climate makes farmers risk-averse. "To this day we are not sure if it will rain or not the next season, so one's harvest is never secure," Walter Godfrey Mjema, another farmer working with Savenije, agrees. "You can say 'next season I will plant maize, and with the new farm management knowledge I will get a better yield, and with this I will buy some new household items,' but then it simply doesn't rain."

Despite the worries that sexy biotechnology is getting all the cash at the expense of researchers such as Savenije, interest in agronomy is growing. Both traditional funders and new donors, such as the Bill & Melinda Gates Foundation and Rockefeller Foundation joint venture, the Alliance for a Green Revolution, are now urging the importance of funding agricultural technologies.

According to the UK Overseas Development Institute, government funding of agriculture in developing countries fell by almost half, in real terms, between 1980 and 2005 whereas for overall development it was increasing 250%. But despite these decades of neglect, moves to fund agricultural technologies would be welcome indeed. Namanga Ngongi, president of the Alliance for a Green Revolution, says that they are looking into technologies such as foot-operated water pumps and solar-powered drip irrigation. And Rajiv Shah, director of agricultural development at the Gates Foundation, says they are eyeing management projects "at the farm and watershed level" as well as grants for traditional breeding.

"There is a huge interplay between crop genetics and crop management, and we believe that these approaches are complementary and synergistic," says Shah. "We are still formulating our priorities for these areas." Maybe agronomy and biotechnology will play nice and work together after all, if the donors push for it. ■

Emma Marris is a correspondent for *Nature* in Missouri.

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See Editorial, page 253.
For more on water see www.nature.com/news/specials/water/index.html

Wilting watersheds

Call them basins, catchments or watersheds, they are the level at which people have to share water day to day. In many basins, demand is tight. Farmers upstream and downstream are negotiating over who takes what out of the river or the groundwater, and they are often up against cities and industry. And there are the fish.

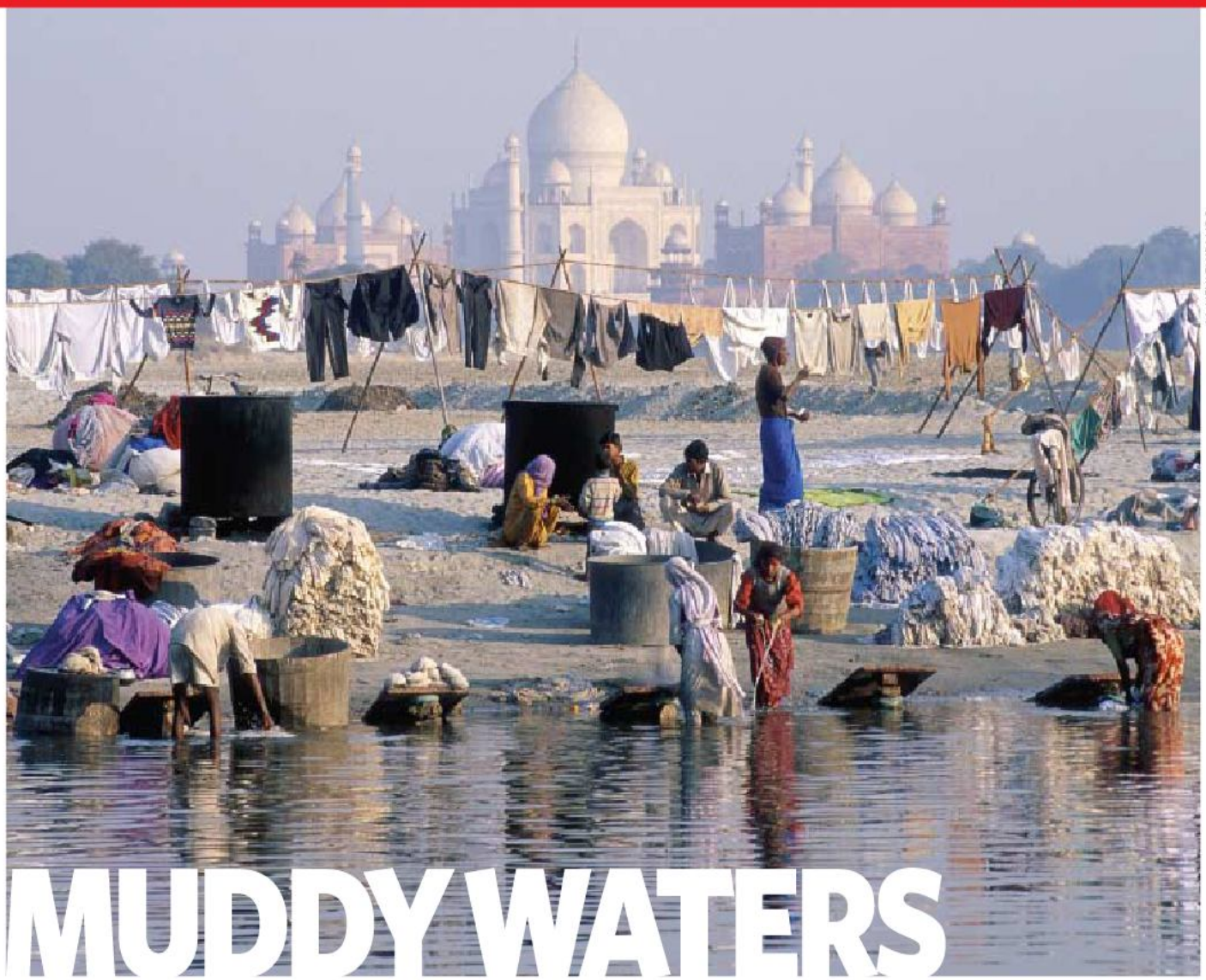
Examples of conflict-ridden basins are ubiquitous. In the United States, for example, the waters of the Colorado River in the south-west are in such hot demand by farmers and cities such as Phoenix that the fight between states, Native tribes and border countries for water rights has rumbled on since 1900.

Suppose a community is very proud that they have ceased over-watering their crops or lined their irrigation channels with plastic. Now imagine that the conserved water formerly sank into the soil and filled wells downslope. Basin-wide, you have no increased efficiency, and downstream you now have hopping-mad well owners.

Many closed basins, such as the Colorado (pictured) and China's Yellow River, no longer flow to the sea. The delta wetlands that once formed their mouths have become empty triangles. The Amu Darya and Syr Darya in Uzbekistan no longer make it to the Aral Sea, which is now half its former size, leaving boats stranded miles from the new shore.

Rivers with no or low flow can be ecological disasters. Scientists are just beginning to determine the minimum water that has to remain for the ecosystem to remain healthy. Fish will need a certain volume of water to swim up to mate, for example. The environment then becomes another competing consumer of water. "The environment is a resource, but it is also a user," says Vladimir Smakhtin, a principal hydrologist at the International Water Management Institute in Colombo, Sri Lanka. "It deserves a fair share of water." **E.M.**

NASA/GSFC/MITI/ERSDAC/JAROS, US/JAPAN ASTER SCIENCE TEAM



M. HENLEY/PANOS

MUDDY WATERS

India's population is growing, and its water supplies are not keeping pace. Can an ambitious scheme to connect up the country's rivers slake the nation's deepening thirst? **Daemon Fairless** investigates.

As it oozes through the city of Delhi, the holy river Yamuna is a vile tract of faecal sludge. From its foul-smelling banks, fishermen cast their nets and haul in a few surviving spiny fish. A group of young men dive, retrieving coins lodged in the river's silty bottom. And nearby, a man empties a burlap sack of ashes — the dusty remains of a family member — into the slime. This 22-kilometre stretch of water is one of Asia's most polluted: 3.5 billion litres of sewage, much of it untreated, makes its way into the river every day.

Of more concern than the quality of Delhi's water, however, is the availability of water at all. Even in the better neighbourhoods, it is common to go several unpredictable hours per day without supply. When the water does flow, up to 40% of it is lost through leaky pipes. Because of the city's notoriously poor municipal water supply, about one-quarter of Delhi's 15 million people rely on privately-dug wells, a practice that itself is lowering the water table.

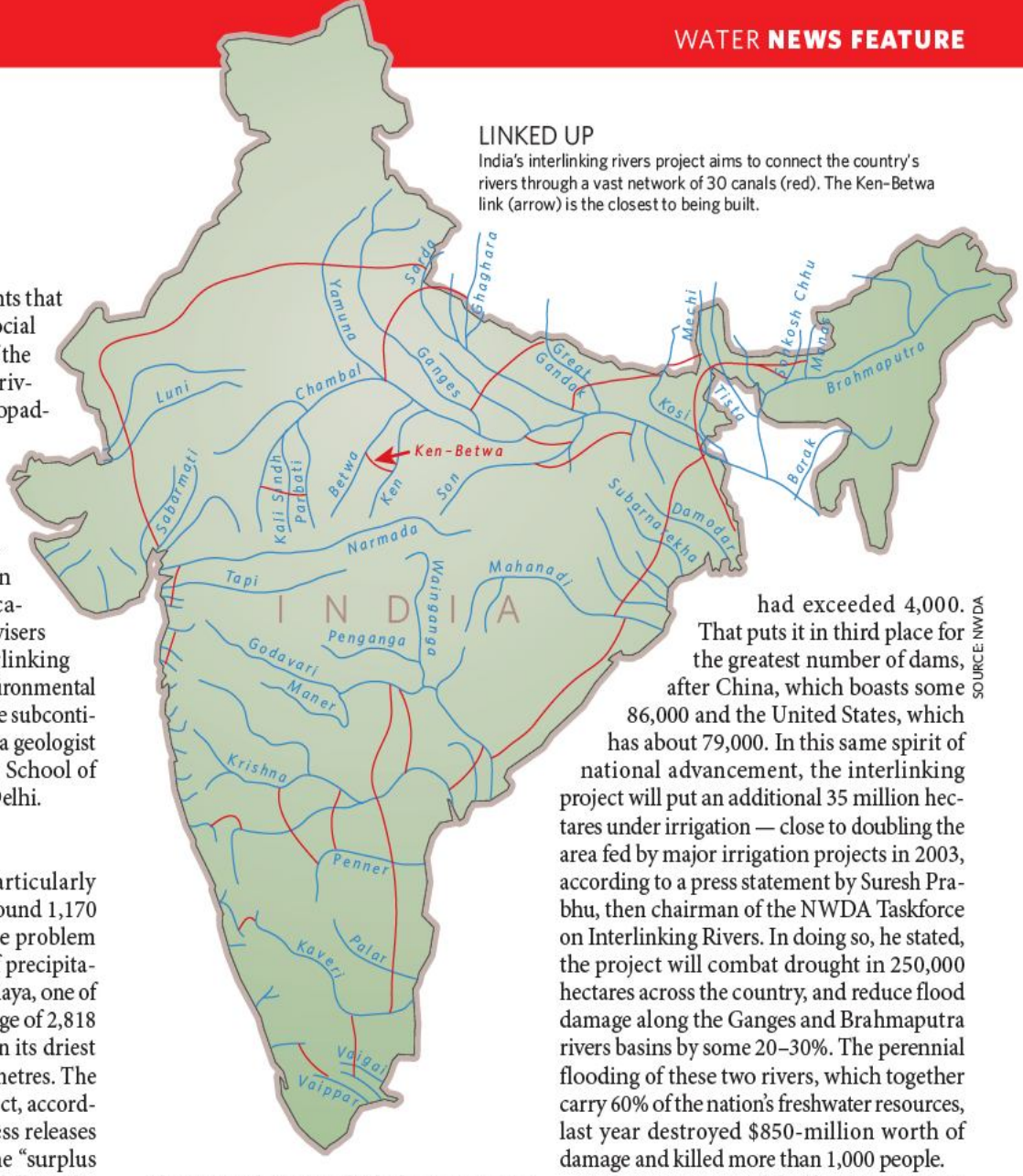
Delhi is a harbinger of the country's looming water crisis. Today, India has enough water to provide each of its citizens with just over 1,800 cubic metres each year — enough to cover the 1,700 cubic metres each individual is estimated to need annually both directly, for drinking and hygiene, and indirectly, to grow the food that person consumes. But as the country's population continues to grow the demand for water will rise. Most of the demand will come from agriculture: irrigation already accounts for more than 90% of the country's freshwater consumption, and much of it is channelled to thirsty crops such as rice. Over the next 20 years, the World Bank predicts that India's per capita water availability will drop below 1,000 cubic metres, well below the 1,700 cubic metres deemed necessary for survival. By the middle of the century, when the population is expected to stabilize somewhere between 1.5 and 1.8 billion, the United Nations Environmental Programme estimates that India will require 30% more water than it can currently supply.

Already farmers in the arid western states such as Rajasthan and Gujarat as well as some of the northeastern states, including West Bengal, are running into trouble as the water table drops from over use. In Delhi too, problems are exacerbated by the demands of agriculture — upwards of 85% of the water from the Yamuna basin has been redirected for irrigation, reducing the river's capacity to recharge the city's natural aquifers. Yet the country's water is likely to be diverted further in future, if the country's planners get their way.

Diversionary tactics

Confident in its ability to find an engineering solution to every problem, India is considering an ambitious plan to link the majority of its major river basins through a vast network of canals, diverting billions of litres from the country's more water-rich river basins to those that are water-deprived. Directed by India's National Water Development Agency (NWDA), the first canal of the interlinking rivers project may get underway as early as this year.

What is not clear, however, is whether the solution will improve the situation. Very little information is publicly available on the scheme's potential environmental impact or on the true extent of the predicted water



LINKED UP
India's interlinking rivers project aims to connect the country's rivers through a vast network of 30 canals (red). The Ken–Betwa link (arrow) is the closest to being built.

SOURCE: NWDA

shortage. "There are no documents that openly establish the economic, social and environmental justifiability of the proposal for [the interlinking of rivers]," according to Jayanta Bandyopadhyay, a specialist in development and environmental policy at the Indian Institute of Management Calcutta and a member of the National Civil Society Committee on the Interlinking of Rivers in India (NCSCILR), a group of academics, researchers and policy advisers who oppose the plan. "The interlinking plan has the potential to wreak environmental havoc on a scale so far unseen in the subcontinent," says Vedaraman Rajamani, a geologist at Jawaharlal Nehru University's School of Environmental Sciences in New Delhi.

Wasted water

India is not, at first glance, a particularly water-poor country. It receives around 1,170 millimetres of rainfall a year. The problem lies in the variable distribution of precipitation: whereas northeastern Meghalaya, one of India's wettest states, gets an average of 2,818 millimetres per year, Rajasthan, in its driest years, can get as little as 100 millimetres. The logic behind the interlinking project, according to the few documents and press releases that are available, is to capture the "surplus water" that otherwise flows wastefully to the sea, and reroute it to where it is needed for irrigation and hydroelectric power.

The idea of linking rivers dates back to the mid-nineteenth century, when Arthur Cotton, a British general and engineer, proposed a series of canals for inland transportation. The current reincarnation came about after a 2002 battle in the supreme court between the states of Tamil Nadu and Karnataka over access to irrigation waters from the Kaveri River. As part of their ruling, the judges mandated that the interlinking rivers project — little more than an NWDA research concept at the time — be completed by 2012. The decision seemed to be largely oblique to the legal case in question and left observers puzzled. "It was a big blow for many of us that the court intervened in this way," says Yoginder Alagh, former minister of both Power and Planning and Science and Technology and a member of the NCSCILR. The Ministry of Water Resources, which controls the NWDA, did not respond to *Nature*'s repeated requests for an interview about the interlinking project.

> **"There are no documents that openly establish the justifiability for interlinking rivers."**
> — Jayanta Bandyopadhyay

The unusual way in which the project was mandated, and the sometimes impenetrable nature of India's government bureaucracies, mean that noone is clear precisely what will be involved, or when. But overall, the proposal is to link 46 rivers from most of the country's major river basins through a 10,000-kilometre long network of 30 canals, several of which will intersect with more than one river (see 'Linked up'). The project, which is estimated to cost about US$200 billion, also includes the construction of 32 major dams. "If undertaken, it would be the single largest engineering project anywhere in the world," according to Ashok Khosla, chairman of the Development Alternatives Group, a non-profit organization based in New Delhi that opposes the plan.

The vast scale of the project is a source of some pride. Jawaharlal Nehru, India's first prime minister, famously referred to large multi-purpose dams as the "temple[s] of modern India". At its independence in 1947, India had only about 300 major dams; by 2000, the number of these modern temples had exceeded 4,000. That puts it in third place for the greatest number of dams, after China, which boasts some 86,000 and the United States, which has about 79,000. In this same spirit of national advancement, the interlinking project will put an additional 35 million hectares under irrigation — close to doubling the area fed by major irrigation projects in 2003, according to a press statement by Suresh Prabhu, then chairman of the NWDA Taskforce on Interlinking Rivers. In doing so, he stated, the project will combat drought in 250,000 hectares across the country, and reduce flood damage along the Ganges and Brahmaputra rivers basins by some 20–30%. The perennial flooding of these two rivers, which together carry 60% of the nation's freshwater resources, last year destroyed $850-million worth of damage and killed more than 1,000 people.

Mukuteswara Gopalakrishnan, secretary-general of the New Delhi-based International Commission on Irrigation and Drainage and former coordinator of the Taskforce on Interlinking Rivers, says that the project could also supply about 34,000 megawatts of hydropower — roughly doubling the current level of hydropower, which lies at just over 25% of the country's current electricity needs. "This is the kind of project any developing country would like to have," Gopalakrishnan says. Government proponents have also argued that the scheme will be beneficial to river ecology because waterways that typically shrink in the dry season would have water pumped into them year round.

Superficial information

The NWDA has published feasibility reports for 14 of the 30 proposed canals on its website. The reports are the only government-supplied data made publicly available on the project and they detail the pros and cons of each proposed canal. They contain few verifiable data and do not cite any research studies; the sections that address putative ecological impacts do little more than state the total area to be submerged by damming, estimate the number of people

M. MOHANAN/ICID

"This is the kind of project any developing country would like to have."
— Mukuteswara Gopalakrishnan

that would be displaced and briefly list some of the local flora and fauna.

Unsurprisingly for a scheme of such magnitude and obscurity, the project has many outspoken critics. A key point they raise is that the concept of surplus water used by the project is misleading, because it ignores the essential ecological role played by run-off and flooding. "It's an entirely anthropocentric definition," Rajamani says. "It does not take into account the fact that these ecosystems developed under the constraints of monsoon flooding and periods of drought." Bandyopadhyay and Shama Perveen, also from the Indian Institute of Management Calcutta, made this argument in one of the first public critiques of the project. In a 2004 article in the online journal *India Together*, they lambasted India's central government as having taken a "reductionist view" — one that "ignores the whole set of ecosystem services provided by water".

Proceed with caution

In August 2005, Rajamani published his own opinion piece in *The Hindu*, one of India's leading national newspapers, criticizing the national plan as having an unsophisticated understanding of the subcontinent's hydrological cycle. In the article, Rajamani said that the annual floods the government planned to mitigate were important in removing agricultural toxins from farm land, depositing soil nutrients and recharging groundwater in key agricultural areas. He argued that such a massive re-working of India's rivers is likely to have some unexpected ramifications, including deleterious effects on the nation's coastal ecosystem and even, potentially, alterations in the monsoon cycle. "It was more or less speculation," says Rajamani, "but my point was that to proceed with such a plan without taking such things into account and doing some very detailed studies would be suicidal".

Rajamani's article caught the attention of then president Abdul Kalam, a strong proponent of the interlinking plan. Kalam surprised Rajamani with a phone call in which he asked if there were any data to back up Rajamani's concerns about the monsoon. There were not — but prompted by Kalam's interest, Rajamani invited several Indian Earth scientists to a one-day meeting in Bangalore in October 2005. Little more than an impromptu brainstorming session, it was the first scientific discussion of the project's environmental ramifications outside government walls. It was also one of the last.

The group concluded "based on a simplistic interpretation of presently available data", that attenuation of water flow into the Bay of Bengal due to river diversion could very well affect the volume, duration and spatial distribution of the monsoon cycle[1]. The freshwater run-off that flows from India's east coast forms a 10–20-metre layer of water over much of the northern Bay of Bengal. This blanket of low-saline water is thought to play a part in the formation of the monsoon clouds which, in turn, deposit most of India's freshwater on its landmass. The group's conclusions make sense, says Edward Maltby, a specialist in wetland management at the University of Liverpool's Institute for Sustainable Water, Integrated Management & Ecosystem Research in the United Kingdom. "There are likely to be many other potentially significant consequences," he adds, "such as an impact on coastal and marine ecosystems as well as the functioning of land-based ecosystems."

D. FAIRLESS

"Environmental science is Dalit science — that is, like our untouchables."
— Vedaraman Rajamani

The main point of the report, says Rajamani, was to show that researchers are not being included in the assessment process: but like other public critiques, the report elicited no public response from the NWDA and little independent follow-up research has been done since.

Classified information

The group also criticized the fact that there are no publicly available estimates of river flow from India's Himalayan rivers. Such data are guarded by the government because water access, which is shared with Pakistan, China, Bangladesh, Bhutan and Nepal, is a major source of political tension. These figures are absent from the feasibility reports, making it more or less impossible for outside scientists to estimate how much water would be left flowing in India's rivers if they were to be diverted. This is a criticism shared widely among environmental scientists. "Even today, river-flow data are classified information," says Brij Gopal, chief editor of the *International Journal of Ecology & Environmental Sciences* and a hydrologist at Jawaharlal Nehru University.

Rajamani says that one of his ongoing frustrations is that environmental science in India is more or less ignored by government planners. Whereas disciplines such as physics and engineering are highly respected, says Rajamani, "environmental science is Dalit science — that is, it is like our untouchables: unseen and unheard". As such, he says, river planning and water management have traditionally fallen into the hands of engineers and government technocrats who are not always qualified, nor necessarily concerned with the environmental impact of large-scale water projects. Gopal is even more vociferous in his criticism. "India's environmental science — or at

J. SILBERBERG/PANOS

R. WALLIS/PANOS

It never rains but it pours: vast geographical differences in India's rainfall make it difficult to manage the water supply.

least its wetland science — is driven by policy rather than the policy being driven by science," he says. If there's one source to blame for the current state of India's rivers, says Gopal, "it's the government engineers".

All this debate could prove academic if the project never materializes. No construction has yet begun, although the first of the 30 proposed links — a 230-kilometre canal between the river Ken in Madhya Pradesh and the river Betwa in Uttar Pradesh — is moving forward. The two states signed a memorandum of understanding with the central government in 2005. Gopalakrishnan, who still acts as a government consultant on the interlinking project, says that a detailed project report has been drawn up and that he is fairly sure that it will be approved in the next year.

Dirty business: India's growing population is expected to make water supplies scarce.

S. FREEDMAN/PANOS

Driving factors

This doesn't necessarily mean that all the links will go ahead. After talking with engineers from the Central Water Commission and the NWDA last summer, Kanchan Chopra, director of the Institute of Economic Growth at Delhi University, says she thinks that the NCSCILR's public criticism has created enough pressure to slow the plan down somewhat. "Rational dissent seems to have tempered the government's enthusiasm," says Chopra. "It may be dying a natural death." But Gopalakrishnan says that this is wishful thinking on the part of those who oppose the plan. "It is almost inevitable," he says, that the entire project will go through. Warnings of India's impending water shortage seem to have endowed the project with a sense of urgency.

But some experts say that the water crisis seems more alarming that it really is, partly because accurate estimates of water availability are hard to come by. Hubert Savenije, a hydrologist at Delft University of Technology in the Netherlands, distinguishes two types of fresh water available for use[2]. 'Blue water' — that from rivers, lakes and streams — feeds crops by irrigation. It can be vital during dry stretches in the growing season, but accounts for only about 20–30% of the actual amount of fresh water that enters food production each year. The rest comes from 'green water', the rain water stored in the soil that is absorbed directly by food plants, and which according to Savenije produces more than 60% of the world's staple food production.

> "India's environmental science is driven by policy rather than the policy being driven by science." — Brij Gopal

The problem arises because estimates of water consumption made by bodies such as the United Nations, generally include both blue water and green water. But estimates of water availability tend to ignore the contribution of green water because it is quite a new concept — a gross oversight, in Savenije's opinion. This is a major problem in India, where there are no accurate estimates of green water. The disparity gives the false impression that demand is hugely outstripping supply, he says.

That is not to say that there is no need to manage water better, just that the ways to do it — those that the government is neglecting in its focus on interlinking — might not have to be so drastic. Alagh, who has been heavily involved with planning India's various irrigation schemes, says that increasing the overall water supply will not, as the project's proponents say, boost agricultural productivity. He points out that although increasing irrigation led to an enormous boost in crop productivity during the 1970s and 80s, it has actually resulted in a drop in overall crop yield in the past five or so years, in part because of mismanagement of new irrigation systems.

Alagh says that the solution lies in better management of existing water resources, rather than importing water for irrigation. A simple way to do this is by using large tanks to collect rainwater, which is later supplied to fields during dry periods. Indian irrigation practices could also be made more efficient. A lot of water is lost in evaporation or through drainage from unsealed irrigation canals, and the common practice of flood irrigation is wasteful compared with drip irrigation, which supplies water directly to the plant's roots. But the water used for irrigation is free, so Indian farmers have little incentive to adopt more economical methods.

Savenije for one feels that the Indian government has lost sight of the simplest solution: to grow more crops in the wet areas and ship them to the dry ones, rather than transporting the water itself. That's because crops are many times lighter, and therefore cheaper to transport, than the water used to grow them. But improving food distribution is a formidable challenge because of the lack of local transportation, the poor condition of much of India's rural infrastructure and inequitable rural markets. But, says Savenije, "it is certainly no more of a challenge than reshaping the country's river network."

On the banks of Delhi's holy river, visitors seem oblivious to the sorry state of the country's water. Families visit the ghats along the Yamuna's banks where, for a few rupees, they pay a small boy to swim out into the slime and launch religious offerings of sweet-meats, fruit and flowers downstream on a small straw mat. They sail downstream, to an unknown destination. India, and its entire dwindling water supply, face a similarly uncertain future. ■

Daemon Fairless is last year's winner of the IDRC Nature fellowship.

1. Rajamani, V. *et al. Curr. Sci.* **90**, 12–13 (2006).
2. Savenije, H. *Phys. Chem. Earth B* **25**, 199–204 (2000).

See Editorial, page 253.
For more on water, see http://www.nature.com/news/specials/water/index.html.

Wildlife disease can put conservation at risk

SIR — In their Letter 'Global trends in emerging infectious diseases' (*Nature* **451**, 990–993; 2008), Kate Jones and colleagues reveal that emerging human infectious diseases are becoming globally more prevalent, particularly those originating from wildlife. Even when cases of all other transmission types started to decrease during 1990–2000 compared with previous decades, cases of wildlife-associated human diseases continued their upward trend. The authors highlight the implications for conservation, advocating more monitoring and preservation of areas rich in biodiversity to counter it.

They do not mention the social and psychological effect this proliferation of wildlife-associated zoonoses could have. Such diseases are widely perceived as a threat to humans (see, for example, W. D. Newmark *et al. Biol. Conserv.* **63**, 177–183; 1993). Negative interactions with wildlife tend to stifle support for conservation policies and initiatives. The increasing prevalence of such diseases could stand in the way of the very conservation initiatives that Jones and colleagues are recommending to protect human health.

Widespread disease in wildlife populations could encourage humans to view wild animals as pests, instead of as resources to be protected and enjoyed. Risk-perception research on wildlife-associated zoonoses would confirm the extent to which this shift has occurred. Such research would also identify gaps between the public's attitudes and epidemiological assessments, and would help to gauge the extent of public support for different proactive management plans. This would enable wildlife managers to decide which plans would be the most politically and socially viable, as well as the best ways to inform the public about them.

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Hall and Keynes join Arbor in the citation indexes

SIR — The career of the non-existent author Ann Arbor is well-known to connoisseurs of computerized databases and citation indexes. Usually listed as the last author, she is sometimes credited with the academic degree "MI". Ann is not actually a person, but the city of Ann Arbor, Michigan, home of the University of Michigan. Her 'degree' is a misinterpretation of the abbreviation for Michigan: MI. She pre-dates online computerized databases, and was often listed in the paper edition of *Index Medicus*.

Ms Arbor now has a UK rival in the team of Walton Hall and Milton Keynes. Like her, they are usually listed as last authors. The online database Google Scholar lists them as co-authors of 46 publications, in addition to their solo work (see http://tinyurl.com/386wuo). Walton Hall is actually a building on the campus of the Open University in Milton Keynes. These 'authors' have a useful role to play: they can be used to check the accuracy of the databases and indexes.

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Growth of activism calls for more thoughtful solutions

SIR — Animal-welfare extremism is spreading, as reported in your News story 'Animal-rights activists invade Europe' (*Nature* **451**, 1034–1035; 2008). For example, activists blocked plans to build laboratory facilities in Venray, the Netherlands, using a campaign that included painting threats on the laboratory directors' houses.

Although many people are concerned about animal experimentation, most do not understand the rationale behind these illegal activities, which generate considerable fear in the research community. Researchers respond by wanting to reduce transparency and asking the government to increase repression of activists — following the UK example of stricter legislation.

Today's understanding of the motivation underlying both normal and abnormal behaviour indicates that this response could be counterproductive. A better solution would be to channel frustrations into more constructive activities. The extremists have received positive reinforcement from their success in blocking the Venray plans. Reduced transparency will only increase societal concern, and repression risks exporting the problem (as it did from the United Kingdom to the Netherlands). Worse, as the extremists are motivated by frustration, repression may amplify the problem.

More constructive solutions include the provision of some form of democratic control, and perceived justice, to people concerned about laboratory-animal welfare. Membership of animal-protection organizations and voting for animal-friendly parties have not proved adequate. As with farm-animal welfare, society could opt for alternative routes. For example, people could request information from medical charities on their funding of animal experiments (and see www.rds-online.org.uk). Medical treatments developed through animal experimentation could be labelled, in the way that some food products are labelled with information about animal welfare. Increased transparency and transfer of at least part of the responsibility from the researcher back to society are key to resolving the wider problem underlying animal extremism.

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Readers are welcome to comment at the Nautilus blog, http://tinyurl.com/2dks88.

How genetic censorship would harm everyone

SIR — In your Editorial 'Genetics benefits at risk' (*Nature* **451**, 745–746; 2008), you indicate that the entire scientific and medical community adamantly supports the US Genetic Information Nondiscrimination Act, because it would protect people from discrimination by health insurers or employers on the basis of genetic information. I, for one, do not support this bill.

Better information allows better matching of people and jobs, and of people and insurance policies. The purpose of firms is to produce goods and services efficiently, and information helps to improve efficiency. The purpose of insurance is to manage risk, and information availability lowers risk.

You fear that the use of genetic information by employers and insurers will lead to social inequality — or, in other words, you trust that ignorance will preserve equity and fairness. There are better ways to deal with social inequality than to force ignorance upon workers, employers and insurers. And a better informed, more efficient, wealthier society creates better conditions for everyone to live decent and productive lives, whatever our genetic make-up.

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Qualities of a lone author are beneficial to science

SIR — Mott Greene's Essay 'The demise of the lone author' (*Nature* **450**, 1165; 2007) highlights the proliferation of multiple-author papers over the past century. Although collaborative efforts are essential and properly result in papers with many authors, I believe that funding agencies and institutions should also encourage single-author papers. The effort and initiative required to publish alone suggests an independent and tenacious scientist — both highly desirable qualities in any researcher.

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COMMENTARY

Improving on haves and have-nots

All-or-nothing targets for global access to basic amenities such as drinking water and sanitation are outdated. The time has come, says **Jamie Bartram**, for a more fluid approach.

No one can deny the profound effects that water and sanitation can have on public health. In nineteenth-century Europe, municipalities made unprecedented investments in public drinking-water and sanitation to control outbreaks of cholera, typhoid and other infectious diseases. Their success was recalled by a recent survey in the *British Medical Journal* that voted sanitation the most important medical advance since 1840.

Regrettably, shamefully, 150 years after that 'sanitary revolution', the consequences of poor sanitation remain devastating. A cholera outbreak in Peru starting in 1991 killed 3,000 people in 15 months, costing the economy US$770 million — more than the investment in water and sanitation during the entire preceding decade[1]. Around the world, the toll adds up: inadequate drinking-water, sanitation and hygiene cause around 6% of all diseases[2].

Water and sanitation are therefore frequent targets in development circles. The most comprehensive recent 'agenda for development' — the United Nations Millennium Declaration — includes in its eight Millennium Development Goals (MDGs) and 21 targets halving the proportion of the world population without safe drinking water and basic sanitation by 2015.

From an initial global population of 5.3 billion in the MDG baseline year (1990), this means improving global use of 'safe water' from 77% to 88% and of 'basic sanitation' from 54% to 77% (while keeping up with population growth). But both goals share a basic weakness in regarding every human as either 'having' or 'not having' these key amenities; a formula well past its sell-by date.

The formula hasn't developed significantly, possibly since the 1960s when the World Health Organization (WHO) began reporting on the same simple pass–fail criterion — although between 1990 and 2006 the world population swelled from 5.3 billion to 6.6 billion and those living in urban areas rose from 43% to 49%. There are diverse challenges: lack of basic infrastructure in much of the developing world; ageing and deteriorating infrastructure in transition countries; and new threats from emerging pathogens, chemicals, deliberate acts and climate change.

Counting haves and have-nots has the advantages of simplicity and equity. Tell people that a third of humanity doesn't even have a basic latrine at home and the message is loud and clear. It is simple to present visually (see map, overleaf) and was critical in getting sanitation back onto the MDG agenda in 2002. But although this global counting is simple, robust and easy to present, it also has major limitations, not least by not encouraging progressive improvements.

There is no real incentive for nations either near the top or the bottom of the international spectrum to tackle their water and sanitation challenges — because the current 'reward structure' is not set up to recognize the range of steps they could take to improve health. Countries adopt targets appropriate to their own needs and aspirations, which may not line up with the global pass–fail benchmarks. A traditional pit latrine with an earth floor is ranked as 'improved' by national authorities in Zimbabwe, although without a solid floor slab it does not score in global monitoring. Recently, I was challenged by a minister from one of the countries of the former Soviet Union, which needed to improve its drinking water and sanitation. It wanted to be part of the MDG effort but couldn't find a 'way in' because the definitions meant that it had little left to do.

G. PIROZZI/PANOS

Community latrines are a step towards household sanitation for all.

Even if the MDGs are met in 2015, 875 million people will still be collecting water from distant, unprotected sources and 1.7 billion will not even have a simple latrine at home. Currently, the efforts to maintain, replace and extend water and sanitation will mean that the target for drinking water will almost be met. But sanitation targets will be missed widely — by around 880 million people[3]. However, it is debatable whether or not this discrepancy between water and sanitation 'progress' is real.

The benchmark for sanitation is use at home, whereas for water it is an improved communal source — a protected well or spring, for example. Applying benchmarks that require both drinking-water and sanitation at home would better represent what is needed to protect health and secure social benefits. Sadly, raising the water benchmark to a household level alongside the sanitation benchmark would mean missing both targets.

Now is the time to debate new international indicators to stimulate action. Agreement won't come rapidly and the underpinning evidence needs strengthening. For a revitalizing platform to be in place by 2015, we need information

beforehand. That way, we know the baseline we are starting with rather than adopting a retrospective baseline as the MDGs did, effectively forcing continuation of an established system that, frankly, was already looking tired.

Smarter targets

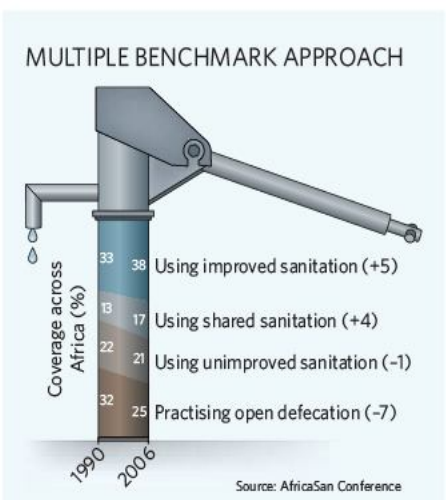
So, what would a better system look like?

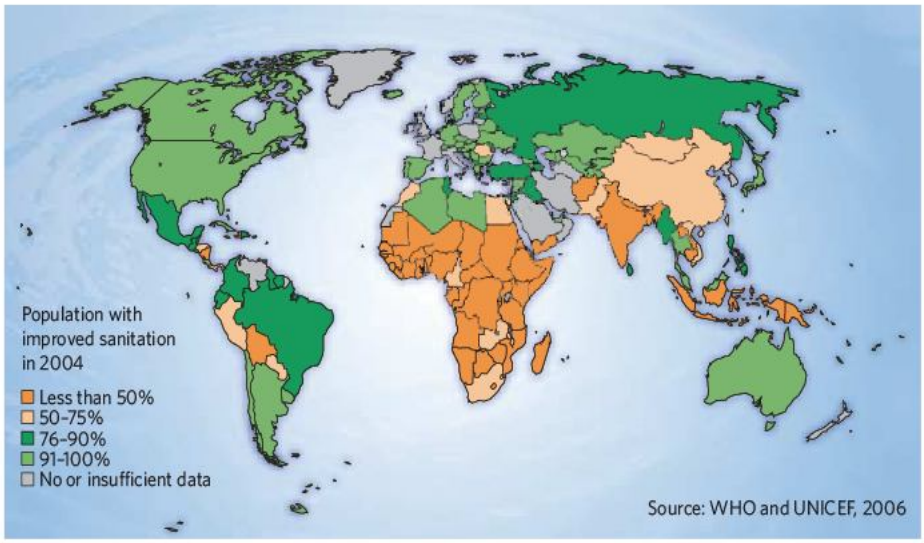
First, it would be relevant to all. The pass–fail criterion is a straightjacket that alienates worthwhile efforts. On the sanitation front, we need a system that recognizes not only a latrine at home but also successful efforts to make shared or even public facilities work in fast-growing, peri-urban areas (where the city meets rural). On the water side, the benchmark of a protected well within a 30-minute walk needs to be supplemented by another where water is available at home.

Overall, we need a sequence of benchmarks reflecting individual steps and potentially different routes to improvement. Such a sequence would reward efforts at all levels. In any one country, some of the population would have reached a higher benchmark than others, so assessing progress between benchmarks for its lowest quartile population could help maintain focus on the most needy.

At least one step towards multiple benchmarks was taken in a report at the AfricaSan Conference in Durban, South Africa, in February (see 'Multiple benchmark approach'). It describes a far more optimistic picture than the simple haves and have-nots categorization — showing open defecation and 'unimproved' facilities in decline and other categories increasing. When extended to water, as the WHO and United Nations Children's Fund (UNICEF) now intend, it will be a real step forward.

Second, a better system would have a firm grounding in health, well-being and livelihoods. Present benchmarks for water availability at a community level and sanitation at a household level, probably reflect what was thought achievable in the rural focus from the 1960s to the 1980s. In fact, the benefits to health and household economy of a distant but protected drinking-water source are very limited, whereas there are large benefits when water is available in every household — in hygiene, productivity and time saved.





Sanitation targets set by the United Nations will be missed by a large margin.

The evidence base for the health gains from potential sanitation benchmarks remains appallingly weak. Public toilets may be considered a key intermediate step and a means to ensure at least some dignity and safety. But some speak of dangers, especially to women, of rape and assault; and poorly maintained facilities are themselves a danger to health.

Third, an improved monitoring system would 'overlay' evidence onto the sequence of benchmarks, although doing so would not be universally popular. For example, if we were to correct statistics on drinking water to account for the fraction of households where it is unsafe, then progress towards the MDGs will appear far more modest. But taking into account the health benefits of treating unsafe water at home and storing it safely would improve health significantly and justify overlaying. If flush toilets were 'marked down' because they discharge untreated wastewater into a nearby river rather than to a treatment facility, then the status in terms of MDG rankings of some middle- and upper-income countries would also drop.

"The pass–fail criterion is a straightjacket that alienates worthwhile efforts."

We will need to invest in tools to collect information on these overlays. Often, those available are costly and inappropriate. Testing water safety — for example, to ascertain faecal pollution — is unachievable in many communities worldwide. We will need to rethink our approaches, as is being done by the Aquatest initiative of the University of Bristol, UK, on the technology needed to make testing achievable in even very low-resource settings. Even without testing a preventive approach, focusing on actions to ensure that water is safe, provides real insights.

Fourth, we need to challenge the perception of households as the only place where people drink water, go to the toilet or wash their hands. An improved monitoring system will recognize that safe water and sanitation in schools, workplaces, hospitals, markets and other public places are also important. There are real grounds for concern, here. One recent report cites half the hospitals of Tajikistan being without water[4].

Fifth, we need to recognize that sanitation protects health best when practised by all. The benefits of sanitation to a household are limited if other community members defecate in the open. So we need to note whether a household has sanitation, and also the sanitation status of the community it is in. And finally, hygiene offers special challenges, with no target or indicator, and none in sight.

The MDG targets for drinking-water and sanitation represent a very limited ambition — leaving many millions without the most basic needs for health protection and development even if they are met. Improved targets would recognize progressive improvements and reflect the range of actions that contribute to health and well-being. They would better align with programming needed for stepwise improvements and help ensure that commitment and momentum are not lost after the end of the MDG period in 2015.

The benefits of clean water and somewhere to defecate have been valued at 3 to 34 times their cost[5]. Isn't the potential for the most important medical advance of the next 150 years worth a better investment? ■

Jamie Bartram is coordinator of the water, sanitation and health programme at the WHO's headquarters in Geneva, Switzerland.

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2. *A Snapshot of Sanitation in Africa* WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (2008).
3. *Unravelling Sanitation Practices* (WHO Geneva & UNICEF New York, 2008).
4. www.euro.who.int/emergencies/fieldwork/20080208_1 (2008).
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COMMENTARY

The energy challenge

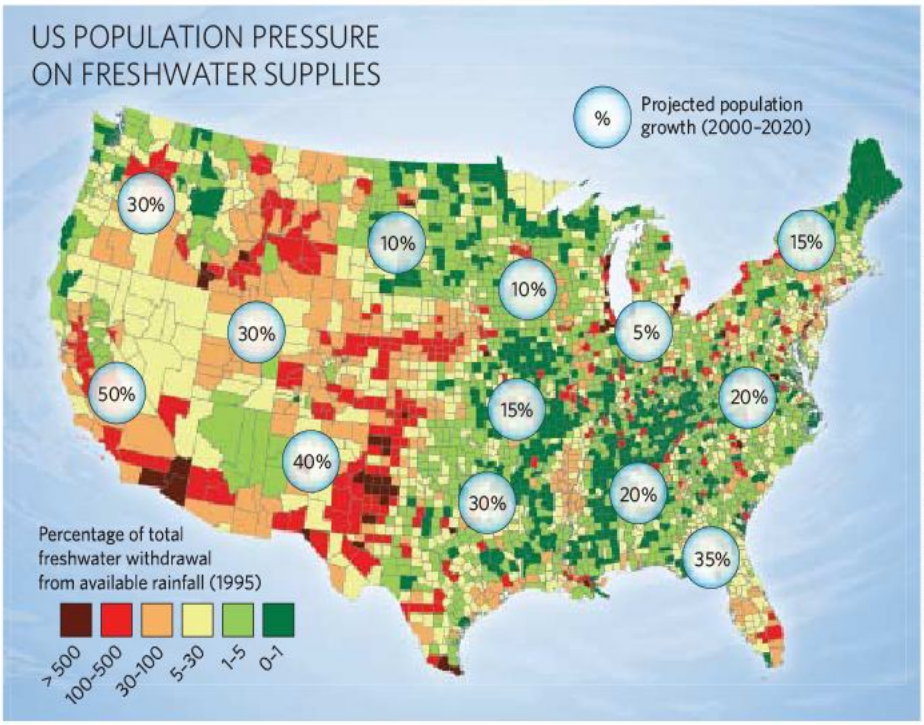
Global energy consumption is expected to grow by 50% by 2030, squeezing already scarce water resources. **Mike Hightower** and **Suzanne A. Pierce** recommend ways to integrate water and energy planning.

In January 2008 at the World Economic Forum in Switzerland, United Nations Secretary General Ban Ki-moon urged business and political leaders that the looming crisis over water shortages should be at the top of the global agenda in an effort to prevent conflicts over the growing scarcity of freshwater supplies. By 2025, more than half the nations in the world will face freshwater stress or shortages, and by 2050, as much as 75% of the world's population could face freshwater scarcity[1].

This growing international water crisis is forcing governments to rethink how they value, use and manage water, especially because economic development hinges on water availability. Drinking-water supplies, agriculture, energy production and generation, mining and industry all require large quantities of water. In the future, these sectors will be competing for increasingly limited freshwater resources, making water-supply availability a major economic driver in the twenty-first century.

In a water-scarce world, regulations, policies and infrastructure development must adapt to the reality that adequate freshwater supplies not only have health and social benefits, but economic benefits as well. Failure to do so will lead to stunted economic growth, inequitable development, and possibly regional conflicts. Yet despite this, water investment as a percentage of gross domestic product has dropped by half in most countries since the late 1990s.

Unfortunately, most nations also suffer from a fragmented approach to water management. In the United States, more than 20 different federal agencies have responsibility for various aspects of water policy, and with a few notable exceptions (the Netherlands and Israel) the situation is similar elsewhere. Under this fragmented approach, integrating water policies and management across several sectors is easier said than done. In many cases, government departments have policies or regulations that are at odds with each other, such as environmental regulations that severely restrict the re-use of domestic waste water. These conflicts reduce, and in many cases actually discourage, coherent approaches to regional water management. As population and economic growth put pressure on local resources — from water to energy and food supplies — a coherent approach is key.



SOURCE: ELECTRIC POWER RES. INST./NATL ENERGY TECHNOL. LAB./US CENSUS BUREAU

Contemporary water planning is often focused on freshwater supplies, but future planning must consider the availability, quality and efficient usage of all water resources in a region to ensure future needs can be met. By using innovative treatments, such as advanced membrane-separation technologies, non-traditional water sources including waste water, brackish groundwater, sea water and extracted mine water can be treated and substituted for freshwater to meet demands that do not require drinking-water qualities. This increases the 'water capital' or the total usable water in a region, providing alternative water supplies to meet growing water needs.

The cost of water

In the past, the cost of freshwater in developed nations has been too low to concern users. For example, water costs in the United States, Canada, South Africa, Australia and western Europe range from less than US$3 to $9 per thousand gallons. But the growing scarcity of freshwater has started to raise prices. Therefore, managing and using all available water capital might both limit future water costs and maximize the health, social and economic benefits of water use, thereby increasing freshwater 'productivity'.

> "The energy sector will find itself in competition with other water users."

To some extent this is already happening: in the United States, waste water reuse is growing at 15% per year, driven by water scarcity and higher prices for freshwater in some regions. There are other, cheaper ways to increase water productivity, such as improving water conservation and efficiency. But water reuse can help to expand these traditional approaches by matching the quality of water supplies to needs, and substituting non-traditional water for freshwater where appropriate. In the energy sector, this approach includes using domestic waste water for power-plant cooling, or even using air instead of water. A less fragmented planning strategy would identify emerging water demands from all sectors, and better match available water resources — of varying quantity and quality — with these demands.

In the developed world, water conservation and efficiency in many sectors, including agricultural irrigation, domestic and industrial water use, and waste-water reuse, have all increased 'water productivity' and augmented

freshwater supplies. But the rapid rates of global population and economic growth will challenge water planners and managers to make sufficient gains in productivity to meet the growing water needs of all sectors.

One area where improvements in water use and productivity are essential is the energy sector. In most countries, irrigated agriculture accounts for most freshwater use (as much as 70–80%), far higher than drinking water and domestic consumption. But the energy sector is catching up fast. In 2000, US electricity production accounted for 39% of national freshwater withdrawals, roughly the same as for irrigated agriculture[2]. By 2030, the Energy Information Administration expects US electricity demand to grow by roughly 50% — in line with projections for global energy consumption — placing an additional burden on freshwater supplies.

The energy sector is one of the biggest users of water.

S. GALLUP/GETTY IMAGES

Unfortunately, freshwater withdrawals already exceed precipitation in many parts of the United States, with the worst shortfalls often in areas with the fastest population growth (see map). In the past the United States has been a champion dam builder, with more than 79,000 dams in the country today, but few new large reservoirs have been built since 1980. As a result, surface water supplies have been level for 20 years, and groundwater supplies are dropping at rapid rates.

Already, freshwater concerns are starting to affect US electric-power generation. The current severe drought in the Southeast has threatened the cooling water supplies of more than 24 of the nation's 104 nuclear power reactors. Not surprisingly, proposals to add additional thermoelectric power plants in the region are meeting increased public resistance.

This is very much a global problem. A severe drought in France in 2003 caused the loss of up to 15% of nuclear power generation capacity for five weeks and a loss of 20% of their hydropower capacity. Similarly, the 2007 drought across Eastern Australia raised concerns over water supplies and electric-power reliability. In the future, the energy sector will find itself increasingly in competition with other water users for limited freshwater resources.

This challenge is increasingly being recognized. A Department of Energy (DOE) report to Congress on energy and water interdependencies, which was initiated in 2005, recommended that the United States should carefully consider energy and water development and management so that each resource is used according to its full value[3]. Also, starting in late 2005, a series of DOE workshops focused on the interdependency of energy and water. The workshop participants recommended integrating regional water, energy and infrastructure to reduce freshwater use where possible[4]. The suggested recommendations on research issues will be published shortly.

Some specific suggestions for reducing water usage by electric power generation include: using waste water, sea water or brackish groundwater for cooling and processing instead of freshwater; using cooling technologies that require less water or no water; switching to renewable energy technologies that do not need water for cooling — such as wind and solar electric; introducing technologies to condense evaporation from cooling towers and capture and reuse the water. Although some of these approaches are in use today, the workshop participants identified research and development to help accelerate their adoption across the energy sector.

All these examples, despite saving freshwater and increasing water productivity, have cost or performance penalties that must be considered. Using brackish water or sea water can require the use of special materials that can be costly, or the use of advanced water-treatment technologies such as reverse osmosis to reduce scaling can be energy intensive, and water withdrawal and discharge systems can impact on the environment. Greater reliance on renewable energy systems, because they are often intermittent, can affect the reliability of energy supplies. In the absence of regional cross-sector planning, such approaches often lose out to the status quo.

"The Romans are a model example of how to develop water resources."

For this reason, freshwater 'capital' and 'productivity' concepts, although useful, must be adopted within a system context to ensure that any economic or social improvements are sustainable across all sectors. In this way, the benefits of saving freshwater compared with increased energy costs or reduced energy reliability can be evaluated at a regional scale, whether across multiple states or multiple nations. Although regional water planning has been practised for decades, integrating water, land, energy use and management to optimize economic development and growth is now emerging for several US river basins (Colorado, Columbia and Missouri), the Nile basin in Africa, the Mekong basin in Asia, and the Murray Darling basin in Australia. This integration provides regional policy-makers with the ability to balance energy reliability and costs alongside the economic and social impacts of water use.

Back to the future

Although an integrated approach might seem to require a major shift in current water resource and infrastructure planning and development, the concepts have existed for millennia. The Romans, for example, recognized the importance of safe and reliable water supplies to promote public health and economic development during the Second Samnite War around 310 BC. To address their inadequate and unreliable water supplies, the Roman Senate procured water rights from surrounding areas and commissioned the development of a system of reservoirs, aqueducts, cisterns and community distribution systems to maintain a reliable supply of water. The Romans came to understand the social and economic benefits of adequate water supplies and implemented major water projects across their empire.

By 40 BC, Roman water-management practices had matured to include concepts for water-infrastructure protection and security, watershed management and providing treatments for water resources of different quality or reserving aqueducts for separate purposes[5,6]. The Romans are a model example of how to develop water resources to optimize water productivity to support public health and economic development.

Although some regions have started to incorporate similar approaches, as noted above, too many countries are still stuck with disjointed water policies. The principles adopted by Roman water planners drove infrastructure development — from aqueducts to water delivery and protection systems — that still serve the public well today, some 2,000 years later. There is no doubt that applying integrated resource-management principles could help provide the framework needed to meet future global energy and water needs in a more systematic and sustainable way. ■

Mike Hightower is in the Energy Systems Analysis Department and Suzanne A. Pierce is in the Geohydrology Department at Sandia National Laboratories in Albuquerque, New Mexico, USA.

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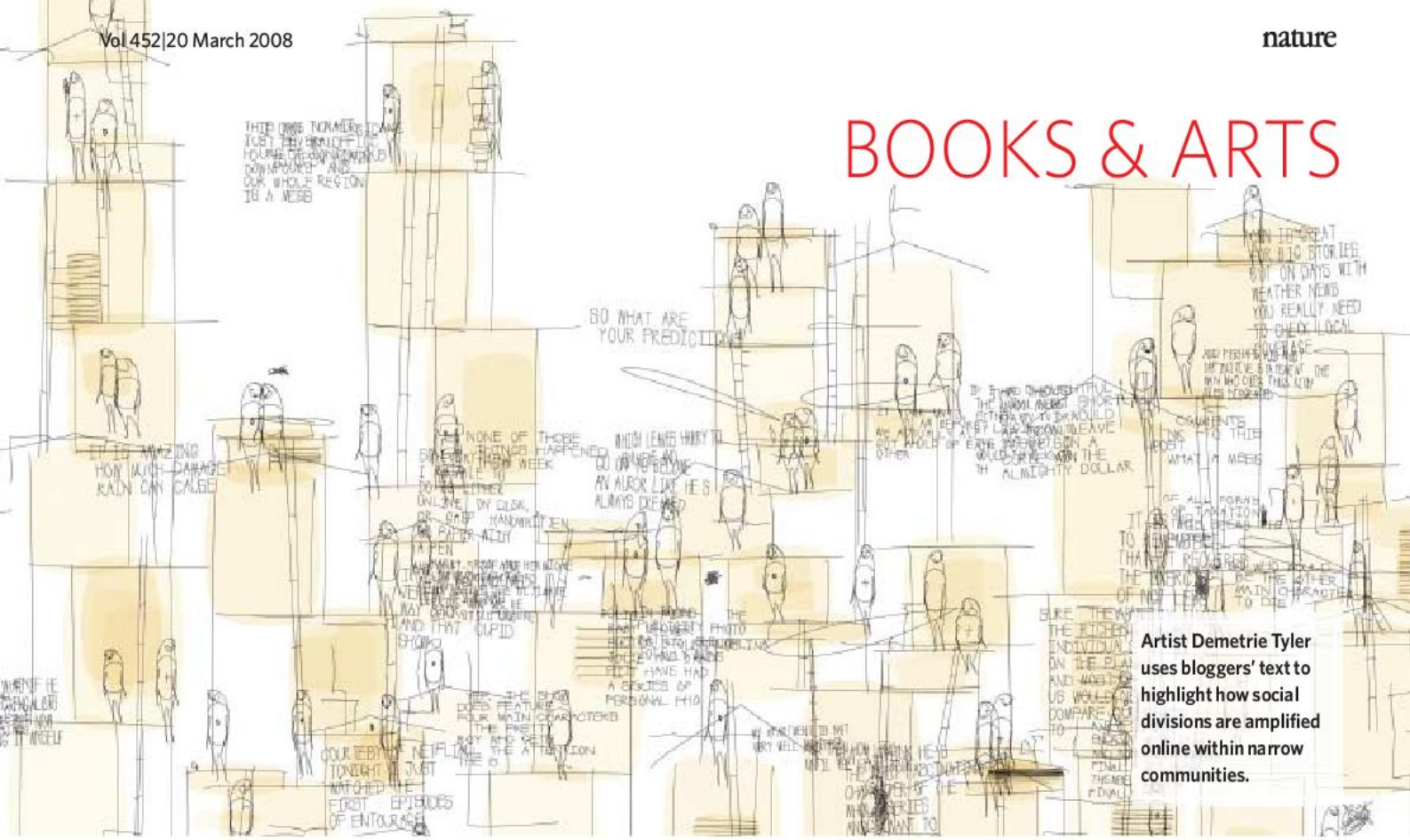
See Editorial, page 253.
For more on water see www.nature.com/news/specials/water/index.html.

BOOKS & ARTS



Artist Demetrie Tyler uses bloggers' text to highlight how social divisions are amplified online within narrow communities.

D. TYLER

Internet as utility?

A sceptic argues that the electricity industry's tale predicts a digital future of diminished privacy.

The Big Switch: Our New Digital Destiny
by Nicholas Carr
Norton: 2008. 224 pp. £15.99

John Browning

Nicholas Carr enjoys annoying information technologists. His first book, *Does IT Matter?* (Harvard Business School Press), looked into the maelstrom of the digital revolution and yawned. His second, *The Big Switch*, now concedes that IT might matter after all, but mostly for the wrong reasons. Computing, Carr argues, will become a boring and regulated utility like electricity, and along the way it will destroy privacy, fracture communities, diminish culture, promote oppression and maybe subjugate the human race to artificial intelligence. Small wonder that the technology magazine *Wired* calls Carr "high-tech's Captain Buzzkill".

The nickname is not entirely an insult. The computing world has an overabundance of buzz, and selective culling is a public service. Carr performs this service best as a reporter, finding information that has been ignored because it doesn't fit IT's messianic self-hype — for example, in the chapter of *The Big Switch* covering blogs.

Internet wisdom hails blogs as a new global conversation: a cross between a model United Nations and an Enlightenment salon whose truths will set us all free. Carr begs to differ. With so much information out there, and so little time, he argues that most people choose to stick with what they know. Instead of reading to broaden their minds, they simply reinforce their prejudices. Carr cites studies from political and computer scientists indicating that liberal blogs link mostly to other liberal blogs, and conservative to conservative. And those who devote their reading time to this belief amplification find it harder to discuss their views with those who think differently. So the allegedly revolutionary new media simply add to the prejudices of the old; more 'discussion' makes for less understanding.

The book is a litany of other predictions of unfortunate and unintended consequences of technology. Few are as thought-provoking as the blogs analysis. Carr never really scrutinizes the forces behind his compendium of depressing anecdotes and dystopian perceptions. The result is a hodge-podge of complaint, unified only by Carr's steadfast belief that computers make the world worse.

This incessant gloom-mongering leaves unanswered the question that is Carr's self-proclaimed theme: what lessons, if any, can the IT world learn from the history of electricity, or vice versa? Carr sketches the career of Samuel Insull, an English clerk who went to the United States as Thomas Edison's assistant. Edison believed that the money in electricity lay in selling generators — the reason he created General Electric. Insull came to believe that the greater fortune lay in selling the generated electricity itself, so he broke with Edison and created the electricity utility Chicago Edison (now Commonwealth Edison).

Similarly, the computing world is now creating some fast-growing utilities. Google provides search facilities to billions; MySpace and Facebook serve as social centres; various Napster descendants share music and other media and information. Only to Carr is it a foregone conclusion that the appearance of such utilities means that all computing will be relentlessly commoditized and served up by giant companies.

In his mighty leap to this conclusion, Carr sails over the fact that the various computing utilities are all very different and don't look much like the electricity industry. Electricity provides a single product, measured in watts and volts, through centralized facilities that offer increasing returns to scale. (Larger producers of electricity traditionally generate it more cheaply than smaller ones, resulting in a tendency for monopoly, which is why it is heavily regulated.)

Computing utilities, by contrast, sell a variety of different and specialized products: ranging from 'relationship-management' software for sales support (for example, www.salesforce.com) to bulk data storage (such as Amazon's S3 service, which provides online storage for the data underlying many large websites). It is not clear that success in one IT field leads to advantage in another, or that the largest player in any given sector has any lasting advantage over smaller rivals — and certainly nowhere

near as great an advantage as an electric utility that owned the wires to its customers and thus controlled access to the market. Equally important, the means of production varies as much as the computing services produced. At one extreme, the peer-to-peer networks (famed for music and video sharing and now bringing massed computing power to problems such as protein folding and code breaking) require each consumer to be a producer too, which is the opposite of monopoly.

Frustratingly, Carr fails to ask whether or how technology itself might have altered the economics of electricity and industries like it. The telephone industry used to look a lot like the electricity industry, dominated by giant, regulated suppliers providing a commodity service. This situation is no more — and computing technology is largely to blame. Networked computers enable robust, distributed control of complex systems. Post-Internet, introducing a new service or a new competitor no longer requires an overhaul of the giant switches at the core of the network. Regulators permitting, innovators can just plug in and play.

Many in Silicon Valley, California, are now debating whether or not technology will do for the electricity grid what it has done for the telephone network. More and more households and companies are generating their own power, sometimes for ecological reasons, sometimes to take advantage of local sunshine, wind or water. Thanks to increasingly intelligent switching, many of these wind turbines, micro-hydro schemes and solar cells are linked back into the grid to compete with the giants of coal and nuclear power. This is also a potential revolution — running precisely opposite to the one Carr predicts.

The most puzzling, and disappointing, thing about Carr is that he remains a sceptic who makes little effort to question what he has read or been told. He just denounces it. Carr believes the conventional wisdom of both the computing and the electricity industries, but he wants everyone to know that the futures they predict won't be as much fun as they say they will. Patient analysis could have made a fascinating book. Yes, too much optimism can be blinding. But so can too much pessimism. ■

John Browning is a journalist based in London who has written extensively about technology, economics and the Internet.

FILM

Water policy in the can

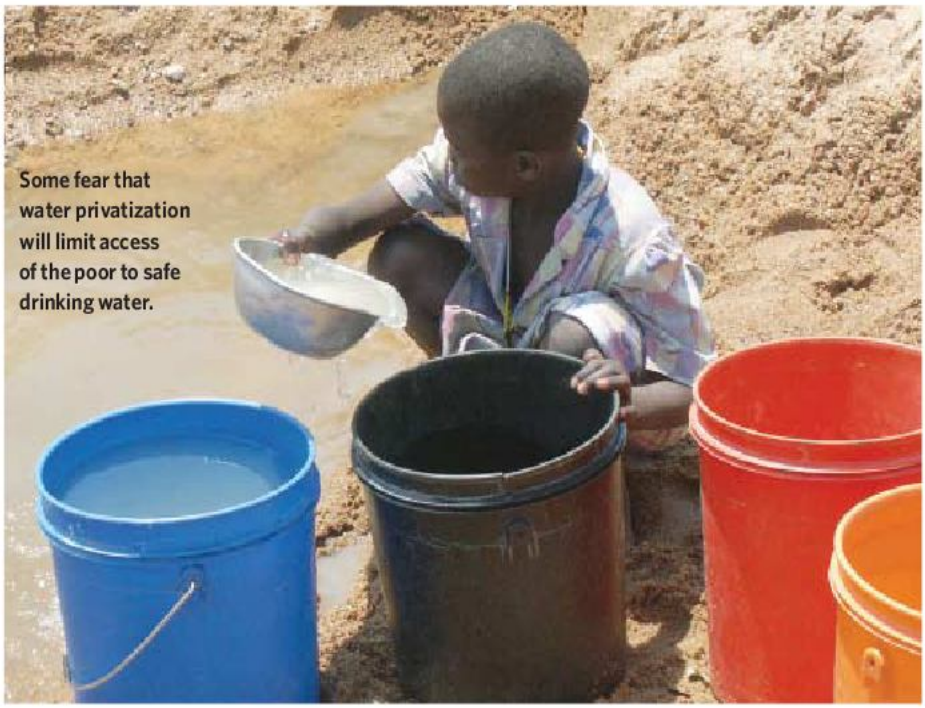
Emma Marris

Many people regard access to safe drinking water as a human right. Yet some fear that the switch from state-run utilities to private ownership will lead to a world where water flows towards the rich as surely as it flows downhill, and where the poor, especially in the developing world, will be left thirsty.

Siding firmly against privatization of water resources, director Irena Salina's documentary *FLOW: For Love of Water* argues that profit-making is intrinsically incompatible with the United Nations Millennium Development Goal to "reduce by half the proportion of people without sustainable access to safe drinking water". Her film, which opens this week, uses case studies of privatization at its ugliest to excite moral outrage.

Some tales are developed more fully than others. Among the most effective is one that focuses on Ashok Gadgil, a researcher at Lawrence Berkeley National Laboratory in California, who as a child watched five cousins die from waterborne diseases. He was moved to invent a cheap device that uses ultraviolet light to disinfect water. The gadget now filters the water of half a million people in India. Similarly coherent is the segment on Michigan citizens battling a water-bottling plant run by the multinational food and beverage producer Nestlé that they feared was turning their creeks into drained mud flats.

Other stories are too big for one film. The section on current scandals and social uprising against privatized water in Bolivia is confusing without more context and chronology. A look at the community effects of large dam projects seems hurried. An intriguing story of women plumbers in South Africa who secretly reactivate water supplies that have been shut off for non-payment gets short shrift.



S. SPRAGUE/STILL PICTURES

Some fear that water privatization will limit access of the poor to safe drinking water.

One segment at first seems out of place among the tales of waterborne disease, future water wars and poor people deprived of water: the Western superstition that bottled water is safer and healthier than tap water. The revelation that it is not feels trivial until the film mentions an uncomfortable statistic. The United Nations estimates that the cost of providing safe, clean drinking water to the entire planet — US$30 billion a year — is less than one-third the amount that the world spends on bottled water annually.

FLOW is intentionally one-sided. Salina presents no cases in which privatization improved the situation, despite there being a few published examples of local successes among many documented failures, such as fewer children dying in Argentine municipalities that privatized their water.

The movie does find time for a long, rousing climax and a call to action. The audience is asked to sign a petition to establish a new article in the Universal Declaration of Human Rights that reads: "Everyone has the right to clean and accessible water, adequate for the health and well-being of the individual and family, and no one shall be deprived of such access or quality of water due to individual economic circumstance."

FLOW zealously marshals a powerful set of arguments against water privatization. But a few more dry facts and a bit less fluid storytelling would have better served this important topic. ■

Emma Marris is a correspondent for *Nature*.

***FLOW: For Love of Water* is released on 21 March in Louisville, Kentucky, and across the United States later this year (www.flowthefilm.com).**

EXHIBITION

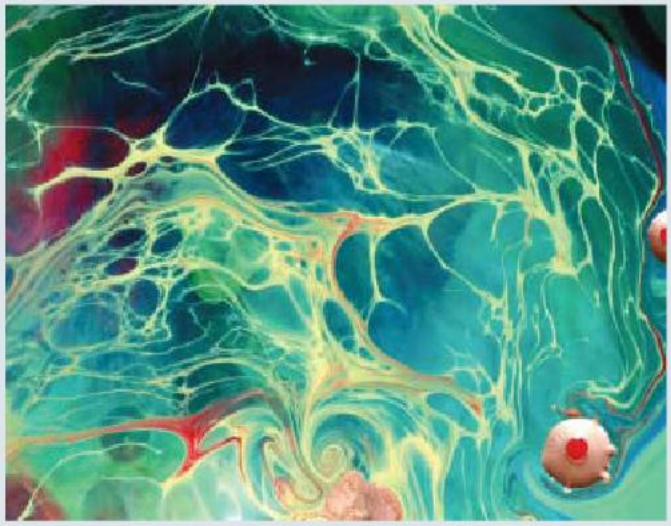
Water works

Nick Thomas

After an injury left her temporarily unable to hold a paintbrush, British artist Pery Burge discovered a new way to produce art while recuperating. She began mixing inks in water and was inspired by the patterns that formed as the ink diffused. Now, the artist, who is based in Devon, UK, focuses on three-dimensional radial spreads — the outward movement of liquid from a central point — and uses time-lapse photography to create a permanent record of these colourful explosions of abstract beauty.

Burge mixes water and ink with droplets of gold paint, oil and the organic solvent xylene in a small, stainless-steel bowl. Complex shapes develop depending on the properties of the ink, such as flow rate and surface tension when the liquids first mix. "I think of myself as a catalyst for nature," says Burge. "I make careful choices about the ink and then watch what happens as the spread develops." She photographs the results in full sunlight and with a flash to highlight the colours. "My aim is to show that nature is the best artist."

Burge feels that the movement of ink through water has aesthetic and scientific aspects, with links to patterns that are commonly found in nature. Some images seem to be alive and organic, reminiscent of unicellular organisms; others look like hexagonal rock formations, crystalline structures or even cosmic phenomena. "Everyone reads them differently," says Burge. "People see figures, landscapes, faces. Your imaginative contribution completes the creative process." ■



P. BURGE

Nick Thomas is associate professor of chemistry at Auburn University Montgomery, Montgomery, Alabama 36124-4023, USA.

Burge's work will be on show from 9–27 April at the National Society Summer Exhibition, Jersey Galleries, London. See www.chronoscapes.co.uk.

Galileo the artist

Galilei der Künstler. Die Zeichnung, der Mond, die Sonne
by Horst Bredekamp
Akademie-Verlag: 2007. 525 pp. €44.80 (in German)

Thomas de Padova

As a young man, Galileo Galilei considered becoming a painter. He acquired extensive knowledge of perspective from Ostilio Ricci, the court mathematician in Florence, who later taught at the Florentine Academy of Design. Galileo was a close friend of the painter Lodovico Cigoli and was in great demand as an art critic, advising Bronzino, Empoli and the cream of Tuscan painters. His trained eye and practice in drawing proved to be extremely useful when Galileo suddenly turned to the study of astronomy at the age of 45.

Galileo was not the first scientist to observe the Moon through a telescope. The Englishman Thomas Harriot did so a few months earlier, in the summer of 1609, and Galileo followed suit, building better telescopes using top-quality lenses. But Galileo did more than that. He saw more. And he drew what he saw, delineating the features of the Moon's landscape, its mountains and craters.

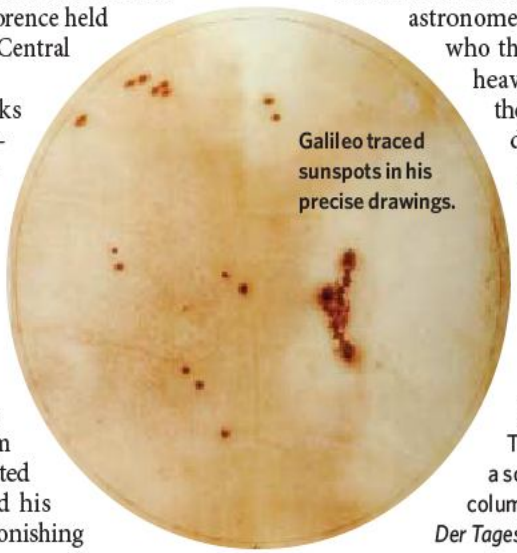
In *Galilei der Künstler* (*Galileo the Artist*), German art historian Horst Bredekamp contends that Galileo's mastery of the modulation of light and shadow made drawing an instrument of learning for the great scientist as well as a method of documentation. Just as Galileo's Moon sketches convinced people — who at the time trusted images more than words — that the Moon was not a perfectly smooth sphere, so Bredekamp's intriguing book succeeds in showing that the act of seeing is itself a powerful tool of analysis.

Bredekamp gained access to unique sources. A few years ago, a previously unknown copy of Galileo's ground-breaking collection of his telescopic discoveries published in March 1610, the *Sidereus Nuncius* (*The Starry Messenger*), was acquired by a United States art dealer. Bredekamp, a professor of art history at Humboldt University in Berlin, suspected that the Moon drawings in this copy were forgeries. Nevertheless, he flew to New York to compare them with the renowned Galileo drawings from Florence held in Italy's National Central Library.

"I spent weeks examining everything meticulously, and finally concluded that they were indeed originals," says Bredekamp. Independent studies by scientists, paper experts and restorers from Berlin and the United States confirmed his view. It was an astonishing and exciting find. Bredekamp believes that these Moon sketches, drawn by Galileo himself, served as templates for the better-known copper engravings in the published *Sidereus Nuncius*, a view that is yet to be corroborated by other experts.

Posterity's judgement of Galileo has varied more than that of any other scientist — books about him have ranged from *Galileo the Martyr* in the seventeenth century to *Galileo, Heretic* and *Galileo, Courtier* more recently. Now the author of *Galileo the Artist* argues for the extent to which the astronomer's artistic talent furthered his scientific achievements, as also illustrated by his discovery of sunspots, detailed in the second part of the book.

Galileo studied these cloud-like structures on the surface of the Sun through drawings he made around 1612. Much as with the Moon, this solar phenomenon had previously been misidentified, this time by the Jesuit astronomer Christoph Scheiner, who thought that they were heavenly bodies orbiting the Sun. Galileo's sunspot drawings, Bredekamp argues convincingly, enabled him to discover that the Sun, like the Moon, is not the perfect sphere that Aristotle had claimed. These pictures alone make the book worth reading. ■



B. HERRENKIND

Galileo traced sunspots in his precise drawings.

Thomas de Padova is a science journalist and columnist at the newspaper *Der Tagesspiegel* in Berlin.

Echoes of time in images of the Antarctic

British landscape artist Chris Drury imaginatively interprets radar pictures taken of ancient ice layers underneath the south pole.

Martin Kemp

We have become accustomed to the idea that, in nature, time frequently manifests itself in layers. We may think of the rings in trees or the ridges in shells, both formed by patterns of unequal growth. Over the longer term, geological stratification provides the most obvious example.

The idea is, in fact, relatively new. Although Nicholas Steno recognized in the seventeenth century that rock strata embodied time, it was not until the nineteenth-century work of Charles Lyell that strata were clearly envisaged as translating directly into eons of time.

With our present urgent concerns about what we are doing to our planet, the stories that are locked into the succession of layers of rock have become more than a simple matter of understanding the deep history of Earthly time. As with much good history, these stories have the potential to help us make informed judgements about the future.

This is particularly true of the patterns of ice and snow accumulation in polar regions, where the single element of frozen water displays, in the most direct way, the regularities and irregularities of temperature-driven processes within the body of Earth.

In December 2006, the British artist Chris Drury travelled to Antarctica to work with the British Antarctic Survey for three months as part of its artists and writers programme. Drury has developed a habit of discerning the analogous nature of organic and inorganic phenomena across different scales. In Antarctica he discovered an awesome subject that did not readily yield to his established practices.

"The ice sheets of Antarctica are an endless expanse of nothingness," he writes. "It's the kind of intense nothingness that both fills and empties the mind." In the face of this sublime vastness, "any mark made by man is like pissing in the wind — irrelevant and gone in the next moment".

He began his time in Antarctica by making sculptural interventions in the physical environment, but became increasingly engaged with the methods that the scientists were using to chart the expanse of time embedded within the thick slabs of ice beneath their feet.

One such method uses ice-penetrating radar beamed from an aircraft to obtain echograms from ice strata that are up to four kilometres deep and 900,000 years old. The echogram data Drury used — the printouts of which can be as long as 20 metres — resulted from four-hour round flights over the polar landscape. Fascinated both by the images themselves and their resonances with other physical phenomena, he printed out small sections on artists' paper, reworking the lines in ink and pencil. Manually and visually, he drew out the sense of flow across the undulating surfaces that demarcated the zones of time.

C. DRURY

Chris Drury sees echoes of landscapes in ice layers.

The data in the original echograms are intended to be read in a specific, analytical way. As an artist, Drury hopes to draw us into seeing the "bigger picture, exploring unusual connections". *Under The Ice, Over the Unknown, detail Flight G23* (pictured) presents a rich opportunity for echoes of an imaginative kind.

My immediate thought was that the illustration looked like a Chinese landscape painting, particularly the monochrome ink paintings characteristic of southern China during the Song dynasty. Then I recalled the 'images made by nature', most notably the 'landscapes' that are visible by sectioning and polishing veined 'hard stones' (*pietre dure*) — a speciality of Florence from the seventeenth century onwards. Drury also saw similarities with echocardiograms, among other things, and superimposed the trace of a pilot's heartbeat onto one of his echogram sections (http://chrisdrury.blogspot.com).

With Drury's artwork, we are in a territory that we can all recognize: that of imaginative projection. Patterns produced by chaotic systems, such as clouds or, as Leonardo da Vinci recognized, in stains on walls, are particularly amenable to such projection. The Chinese painters, for their part, certainly exploited what might be called controlled chance when they let their ink run on the paper.

There is no right or wrong in this kind of reading by visual analogy. It is certainly very different from how scientists read echograms. But it is not necessarily unscientific. It can lead us into thinking about the kind of natural properties that have fascinated artists and scientists alike: time, accretion, flow, pattern, chaos, self-organization, rhythm, layer, scale, organic, inorganic, human and non-human. At the heart of these properties are processes that can be vast and minute, robust and fragile — and beautiful. Our engagement with the processes using every faculty we have available is what stands between us and disaster. ■

Martin Kemp is research professor in the history of art at the University of Oxford, OX1 1PT, UK.

See Chris Drury's artwork at www.chrisdrury.co.uk.

Water — an enduring mystery

Yet another theory of liquid water structure raises questions about interdisciplinarity, drug design, astrobiology, molecular biology, geochemistry and more.

Philip Ball

K. HOKUSAI (1790–1849)/BRIDGEMAN

No one really understands water. It's embarrassing to admit it, but the stuff that covers two-thirds of our planet is still a mystery. Worse, the more we look, the more the problems accumulate: new techniques probing deeper into the molecular architecture of liquid water are throwing up more puzzles.

This guilty secret has myriad ramifications. Water defines the terrestrial environment. It is central to Earth and atmospheric sciences, to biology and to many technologies. The common assumption that water is well characterized has led to explanatory edifices built on shaky ground. The situation is unsatisfactory intellectually and hazardous in practice.

Everyone is agreed that one aspect of water's molecular structure sets it apart from most other liquids: fleeting hydrogen bonds[1]. These feeble bonds that link the molecules constantly break and form above water's melting point, yet still impose a degree of structure on the molecular jumble.

That's where the consensus ends. The standard picture of liquid water[1] posits that each molecule of H_2O is, on average, bonded to four others in a tetrahedral motif. This repeated, constantly reorganizing unit defines a three-dimensional network extending throughout the liquid. This prevailing view comes largely from neutron-scattering studies and computer simulations, and it makes good sense in the light of the unambiguously tetrahedral arrangement of molecules in ice crystals.

In 2004, the latest instalment in a long line of dissension emerged. Lars Pettersson and his colleagues based at Stockholm University in Sweden published a controversial paper in *Science* claiming that molecules in liquid water bind on average to just two others, forming chains and rings[2]. It was a 'string theory of water', if you will. Pettersson's group used X-ray absorption spectroscopy to probe the local environment of individual oxygen atoms.

The interpretation was greeted with scepticism, but the 'string theory' won't go away. The Swedish researchers now claim, in work as yet unpublished, that the conventional tetrahedral structure is not the only way to interpret previous data on water structure from X-ray, neutron scattering and infrared spectroscopy. The 'string' model fits the results too, they say. With physicists and chemists at several institutes in Japan, they have refined their view through X-ray emission spectroscopy.

Water, they now suggest, is a muddle of two different structures. It is a random soup flecked with tiny 'icebergs', each comprising 100 or so loosely cohering molecules. The clusters, they argue, are relatively 'open' and strongly hydrogen-bonded, in keeping with the conventional tetrahedral model. The soup is made from the 'string' structure described in 2004 — denser and with fewer hydrogen bonds.

Such a two-state model would fundamentally change our picture of how dissolved substances behave. Non-polar solutes might be partitioned into the strongly hydrogen-bonded clusters; polar solutes such as ions would swim in the disorderly soup. The consequences would be felt from geochemistry to industrial processing to colloid science. Biologists in particular would need to take heed, because liquid water, widely acknowledged as the 'matrix of life' on our planet at least, is not just a passive scaffold. It has many active roles in molecular biology[3], minutely influenced by its structure.

Right now, many water researchers dismiss the Stockholm work as a storm in a teacup. They think that the aberrant results will turn out to have some mundane explanation, perhaps simply data misinterpretation. It demands considerable suspension of disbelief to accept that the conventional picture of water, assembled painstakingly over the best part of a century, is fundamentally wrong — although stranger about-turns have happened in science.

Regardless of its outcome, this debate is interesting as an illustration of just how difficult it is to understand water, and how widely the uncertainties ripple out. And the

ESSAY

dispute is just one of many. For example, does water form two different liquid phases under extremes of temperature and pressure? How does it rearrange its molecules next to a surface or to accommodate solutes? Is most of the water in cells structurally akin to the pure liquid at all? If or when these spats dissolve, history leads us to expect others to bubble up in their place.

Too anomalous, too strange

The constituency pondering such issues should be far wider than at present. One problem is that even many of those who work on general theories of the liquid state of matter won't go near water: it is too anomalous, too strange. It does not do what liquids are 'supposed' to: it expands on freezing, it is densest in the liquid state at 4 °C rather than becoming steadily denser as it cools; it has an abnormally high heat capacity, odd viscosity, and more. Most of these anomalies are rationalized by the standard tetrahedral hydrogen-bonded network. But it's still not clear how this delicate molecular interlinking translates to bulk-scale behaviour. Computer simulations are often used to explore matter's molecular-scale character, but for water these are notoriously sensitive to how the forces between molecules are modelled.

There's nothing new in a two-state picture of water. In 1892, well before hydrogen bonding was recognized, Wilhelm Röntgen proposed that cold water contains microscopic 'icebergs' in a fluid 'sea'. In the 1920s Henry Armstrong's 'hydrone' theory propagated the idea of long-lived clusters of water molecules, as did Oleg Samoilov's 'interstitial' model in the 1940s. Most recently, the chemist Wilse Robinson in Texas tirelessly promoted the idea that water is a mixture of two forms until his death in 2000.

Such ambiguities allow wilder ideas to insinuate themselves. The polywater affair of the late 1960s stemmed from a claim by Russian chemist Boris Deryaguin and his colleagues to have observed a gel-like form of water in small capillary tubes. Even more outrageous were the experiments on high-dilution biological solutions, conducted by the late Jacques Benveniste and his collaborators in France in the 1980s. These created the notion of the 'memory of water', whereby the liquid can allegedly become imprinted with biomolecular information. Still wheeled out in justification of homeopathy, this improbable idea continues to trade on genuine uncertainties about water structure.

Do these disputes matter to anyone but those involved? One of the strengths of science is that it can operate in a modular manner. We can make a lot of progress in one direction while deeper questions about more fundamental issues remain unresolved. Were that not so, every field would halt until we had a theory of quantum gravity. Chemists can formulate effective models of atomic bonding and molecular structure without knowing nuclear physics; evolutionary biologists need not grasp the chemistry of genetics.

But molecular biology depends inextricably on what water is like at the molecular scale. The iconic view of DNA's double helix, for example, is disingenuous: it is only the molecule's structure in water. In the gas phase, the helix looks as though a child has stamped on it. Hydration changes, such as removing water from the surface of the molecule, can induce switches in DNA conformation. Recent experiments show that the double helix spontaneously unzips when dragged into a non-aqueous solvent, suggesting that the same might happen in a low-water environment. It is likely that nature exploits these properties to manipulate DNA; maybe, for example, hydrophobic cavities in enzymes assist the unzipping that precedes replication.

> "Even many of those who work on general theories of the liquid state of matter won't go near water."

When proteins bind their substrates, an intervening sliver of water must shift out of the way. This process depends on the structure of the confined water. Moreover, many enzyme binding sites have water molecules attached to their hydrophilic regions. Some of these molecules cede their position to the incoming substrate, others stay in the binding site and supply hydrogen-bonding bridges to the docking entity.

Chaos and order

All this entails a subtle balance of energy costs: that of bond making and breaking (enthalpy) and that of disorder changes in the molecular components (entropy). The enthalpy cost depends in part on how many hydrogen bonds the expelled water molecules make in the bulk liquid; the entropy change is also contingent on the degree of ordering there. Estimates imply that, on average, the various costs and benefits of releasing a water molecule cancel out, so small factors specific to each case could tip the scales either way, making binding more or less favourable. A similar fine balance probably governs the important interactions of proteins with carbohydrates.

These delicately poised energetics are crucial in drug design. Here the aim is to engineer good binding between a drug and its target — a small molecule, perhaps, that slots into an enzyme binding site to block it. Some inhibitors of HIV-1 protease, a key target in AIDS therapies, bind via a bridging water molecule. Others have been designed to exclude it. But docking design has so far capitalized little on water molecules because their role is not sufficiently understood or quantified.

The role of water structure in molecular biology is perhaps most apparent in the attractive force that operates between two hydrophobic entities in water. Hydrophobic attraction drives the correct folding of protein chains, the binding of some proteins to substrates, and the aggregation of proteins into functional units and dangerous clumps such as amyloid fibrils. It is one of the key forces in molecular biology. And no one understands it.

Several biochemistry textbooks will tell you that it was explained by Walter Kauzmann in 1959. Kauzmann argued that water becomes more 'highly structured' around hydrophobic solutes, and that the release of some of this water into the relative chaos of the bulk liquid when two such solutes stick together produces a favourable increase in entropy[4].

This is almost certainly wrong. For one thing, the hydrophobic interaction may operate in different ways for small solutes such as methane and for the kind of extended nanoscale surfaces that proteins have[5]. Such surfaces may stick together via a sudden, coordinated expulsion of many water molecules, although whether and how much this happens in real protein folding and association remains unclear. The point is that all such mechanisms depend fundamentally on the structure of bulk water and how that alters near a hydrophobic entity.

Must water's enduring mystery merely induce despair in those who want to know how proteins fold and function, say, or how minerals dissolve or suspended particles clump together? No. Sometimes the details won't matter much, sometimes empirical knowledge and heuristics will suffice. Think of this puzzle more as an exercise in humility. Water reminds us of the dangers of doing science in silos, the risks of leaving apparently tidy explanations unexamined, the importance of not letting ubiquity lead to invisibility, and the recognition that new ways of studying the world can exacerbate as well as dispel confusion. ■

Philip Ball is a consultant editor of *Nature*. His books include *H_2O: The Biography of Water* and *The Self-Made Tapestry: Pattern Formation in Nature*.

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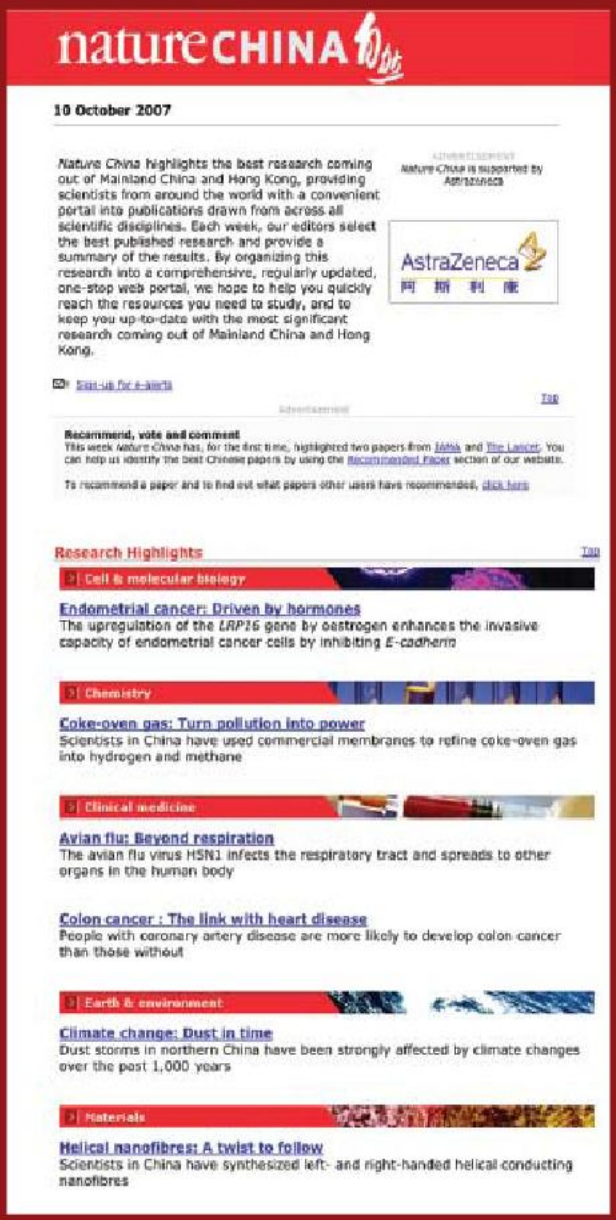

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NEWS & VIEWS

PARTICLE PHYSICS

Song of the electroweak penguin

Michael E. Peskin

An unexpected imbalance in how particles containing the heaviest quarks decay might reveal exotic influences — and perhaps help to explain why matter, rather than antimatter, dominates the Universe.

Elsewhere in this issue, the Belle collaboration, based at the electron–positron particle collider of the high-energy accelerator laboratory KEK in Japan, announces their measurement of an anomalous asymmetry in the decay rates of exotic particles known as B mesons (Lin *et al.*, page 332)[1]. Combined with recent measurements of the same decays from the BaBar collaboration[2,3], a similar experiment at the Stanford Linear Accelerator Center (SLAC) in California, the new finding provides a tantalizing glimpse of a possible new source for a very fundamental asymmetry: the dominance of matter over antimatter in our Universe.

The two great principles of modern physics, quantum mechanics and Einstein's relativity, together imply that every particle in nature — among them the quarks and the leptons, the elementary particles of matter — has an antimatter counterpart with exactly the same mass, and exactly the opposite electric charge. Over the past 20 years, the theories of the weak and strong nuclear forces that have been built up on this basis have passed numerous rigorous experimental tests. The mathematical form of these theories allows little space for interactions that treat particles and antiparticles differently.

And yet the Universe, as far out as we can see, is made of matter, not of antimatter. We see no signals of the matter–antimatter annihilation that would happen on the edge of our local region if only this region were dominated by matter. So did the initial conditions of the Big Bang perhaps contain more matter than antimatter? It is possible. But in inflationary cosmology, the model that has successfully explained the large-scale distribution of mass in the Universe, any such initial asymmetry would have been erased very early on. We are forced to conclude that the current asymmetry has evolved from a symmetric situation since the end of the cosmic inflation that followed the Big Bang. Nature, it seems, treats matter and antimatter differently[4].

In 1973, Makoto Kobayashi and Toshihide Maskawa pointed out that a term could be added to the theory of the weak interaction (which changes one type of quark to another, for example in radioactive decay) to make this force act asymmetrically on matter and antimatter[5]. This difference would appear only if there were at least six types of quark.

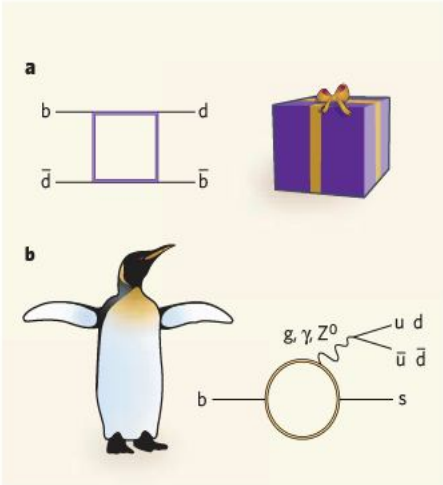

Figure 1 | Weakly decaying. A Feynman diagram represents the time evolution of a particle process (shown here from left to right). **a**, In a standard 'box' diagram of weak quark-mixing interactions, quarks change type by exchanging a pair of particles, for example a heavy top (t) quark and a W boson, the intermediary of the weak force. Here, a $\bar{B}^0$ meson (quark content $\bar{d}b$) converts into a B^0 ($\bar{b}d$). **b**, In a penguin process, the change of quark type occurs via a particle loop, which connects via a boson (wavy line; a gluon, g, gives a 'strong penguin'; a Z^0 an 'electroweak penguin'; γ is a photon) to a further particle. Here, for example, a $\bar{B}^-$ or $\bar{B}^0$ could be decaying into a K^- ($\bar{u}s$) or $\bar{K}^0$ ($\bar{d}s$), plus an additional u or d quark that combines with the u or d antiquark in the B meson. The other end product is a π^0 particle, which can have quark content $u\bar{u}$ or $d\bar{d}$. In both penguin and box processes, the particles represented by the heavy lines (square in **a**, circle in **b**) could be as-yet-undiscovered exotic particles. Recent results from the Belle[1] and BaBar[2,3] collaborations invite the conclusion that penguin processes involving exotic particles are contributing to B-meson decays in their experiments. (The resemblance of the penguin diagram to a penguin is hard to discern. The name originated in a bet between particle physicists John Ellis of CERN and Melissa Franklin of Harvard University over a game of darts in a Geneva bar[13].)

This was a bold prediction, because at the time only three types of quark were known: up (u), down (d) and strange (s). But in the following decades, three more were discovered: charm (c), and the heavy bottom (b) and top (t) quarks. This astounding success led to the proposal[6,7] that specific experiments on B mesons — quark–antiquark pairings in which one of the particles is a b quark or $\bar{b}$ antiquark — could test the Kobayashi–Maskawa (KM) theory directly. The idea, proposed by Pier Oddone, that these experiments could be performed by colliding two beams of different energies, one of electrons and one of positrons (the antiparticle of the electron), motivated the construction of new accelerators at KEK and SLAC. In 2002, both BaBar[8] and Belle[9] reported the first observation of a KM asymmetry in a B-meson decay.

Since then, evidence accumulated by BaBar and Belle, in a data set of more than 1.2 billion B-meson decays, has been used to fix the two crucial parameters of the KM theory to an accuracy of about 5%. Complementary measurements from other processes involving B mesons[10–12] have confirmed these parameters to accuracies of between 10% and 20%. It would seem that we are well on the way to understanding the basis of particle–antiparticle asymmetry in the early Universe.

In fact, we are not. The KM predictions depend crucially on the masses of the intermediate-mass s and c quarks. But the high temperature of the Universe just after the Big Bang makes these masses irrelevant in calculations of the cosmic-matter excess. The degree of asymmetry predicted by the KM model is ten orders of magnitude too small.

So where does this extra asymmetry come from? If we go beyond the standard picture of particle physics, there are many possible sources. For example, there might be new, heavier types of elementary particles beyond quarks and leptons. The search for these exotic particles is one motivation for building the Large Hadron Collider (LHC) which will soon begin operating at CERN near Geneva, Switzerland. Particle–antiparticle asymmetries are much easier to accommodate in the interactions of very heavy particles.

If these heavier particles exist, they could

imprint themselves on the decays of B mesons: pairs of them might be created as short-lived quantum fluctuations that would contribute to the rate of B-meson decay. The kind of processes in which these particles might pop up are represented by so-called Feynman diagrams (Fig. 1). Two types of diagram are important: 'box' diagrams, involving a straightforward two-way exchange of particles with a resulting swapping of quark types (Fig. 1a); and 'penguin' diagrams, in which a new quark–antiquark pair sprouts from a particle loop via an intermediary particle known as a boson (Fig. 1b). The particles in the exchange or in the loop could be known particles, or heavy exotic ones.

Of the processes that have so far provided evidence for the KM theory, most have involved only the simpler box diagrams. None has tested the contribution from the electroweak penguin process — that is, a penguin process in which the intermediary boson is a Z^0, one of the particles that transmit the weak force. This process is relatively rare, but is potentially the most sensitive to new heavy particles.

This is the crux of the latest finding from the Belle collaboration[1]. They find evidence for an exotic electroweak penguin contribution to decays of B mesons into two lighter mesons: a K-meson and a π-meson. This decay receives contributions both from a direct weak-interaction decay with no loop and from a standard penguin process in which the boson is a gluon, the particle responsible for the strong force. The interplay of these two processes leads to a small difference in the rates of particle and antiparticle processes: the rate of the decay $B^0 \rightarrow K^+\pi^-$ is 20% larger than that of the equivalent antiparticle decay $\bar{B}^0 \rightarrow K^-\pi^+$.

The B^0 meson is composed of a d and a $\bar{b}$; the $\bar{B}^0$ contains a $\bar{d}$ and a b. In both of the above processes, the decay is essentially a decay of the b quark or its antiparticle. The lighter d or $\bar{d}$ does not participate. Given this fact, one would expect that replacing the d or $\bar{d}$ in the B meson by the similarly light u or $\bar{u}$ would produce the same asymmetry. But Belle observes that the equivalent decays of the mesons corresponding to those quark compositions, $B^+ \rightarrow K^+\pi^0$ and $\bar{B}^- \rightarrow K^-\pi^0$, have an asymmetry of the opposite sign. Together with the same asymmetries recently announced by BaBar[2,3], the effect has a statistical significance greater than five standard deviations — the 'gold standard' of particle physicists for proof that an effect is real.

Unlike the decays of the neutral B mesons B^0 and $\bar{B}^0$, the decays of the charged B mesons B^+ and $\bar{B}^-$ produce two u quarks or antiquarks. This means that other processes that preferentially produce u quarks rather than d quarks might affect the asymmetry. The electroweak penguin is just such an effect — but to alter the asymmetry, this process must differ from the standard electroweak penguin, which affects the decay rates symmetrically. A contribution from an exotic loop is required. There are admittedly other possibilities that might explain the anomaly in the asymmetry: a direct weak-interaction decay process, the so-called 'colour-suppressed' contribution, also has the required properties. The size of this contribution depends on the quarks involved. In decays of mesons containing the c quark, it is substantial. For the heavier B mesons, however, it is indeed expected to be suppressed.

The new results[1–3] are not conclusive, but they are tantalizing. They might be due to properties of standard b-quark weak interactions that we cannot quite yet estimate precisely, but it is equally possible that this is the first hint of an entirely new mechanism for particle–antiparticle asymmetry. In the next few years, these ideas will be tested, both through the analysis of the huge Belle and BaBar data set, and from the hunt for exotic particles at the LHC. We do not yet know whether it is penguins or even more unusual creatures that produce our Universe made of matter and not antimatter. ■

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PHYSIOLOGY

Brain comes to light

Hitoshi Okamura

To perceive seasons, animals compare changes in day length with the constant cycle of their inner circadian clock. At a molecular level, light signals trigger coordinated gene-expression events in the brain.

To survive, organisms must adapt to the constantly changing conditions of their surroundings. For example, most animals that live at temperate latitudes concentrate their reproductive efforts to times when environmental conditions such as temperature and food availability are optimal for the survival of their offspring. As a reliable indicator of a season, many organisms use day length (photoperiod), which seems to be physiologically encoded in their circadian clock[1]. On page 317 of this issue, Nakao *et al.*[2] shed light on the molecular events that occur in the brain of Japanese quail (*Coturnix japonica*) in response to increased day length. These birds normally breed as the days are getting longer.

The signal response to light is integrated in the brain's hypothalamus, where it enhances the secretion of gonadotropin-releasing hormone (GnRH). This leads to increased blood concentrations of luteinizing hormone and follicle-stimulating hormone, both of which originate from the pituitary gland at the base of the brain. Increased gonadal activity follows[3]. Nakao *et al.* have found that the brain region in which these sequential changes are triggered is the junction between the hypothalamus and the pituitary (Fig. 1). Specifically, it seems that this junction is formed from a part of the hypothalamus known as the median eminence and from the pars tuberalis of the pituitary. The authors also report that the triggering agent is thyroid-stimulating hormone (TSH), or thyrotropin.

The authors found that, when the quail were exposed to long days, two waves of gene expression occurred: the first came 14 hours after the dawn of the first long day, and the second followed four hours later. Gene-expression events of the 'first round' led to a rise in the levels of thyrotropin in cells of the pars tuberalis. It had been thought[4] that expression of the gene encoding the thyroid-hormone-activating enzyme DIO2, located in the median eminence, was the earliest event in the photoperiodic response in these birds. But Nakao *et al.* found that this gene is expressed only in the second round of gene expression.

The median eminence is the gateway through which brain neurons must pass to reach the rest of the body. For example, hypothalamic neurons carrying hormones such as GnRH project their fibres towards, and terminate around, the dense capillary plexus (network) of the median eminence. The median eminence contains specialized epithelial cells called tanycytes (from

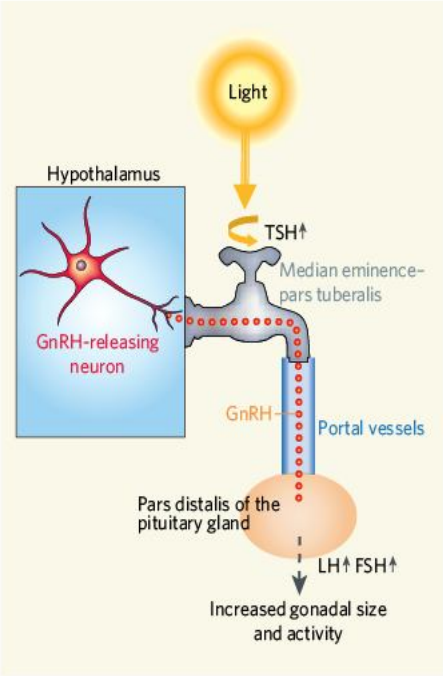

Figure 1 | A light-sensitive hormonal tap. Photoperiodic light signals activate a functional unit at the base of the brain consisting of the region of the hypothalamus known as the median eminence and the pars tuberalis in the pituitary gland. Nakao *et al.* find that thyrotropin (TSH) is released there, leading to the release of the hypothalamic hormone GnRH from neurons that project to this 'tap'. GnRH enters the portal vessels and promotes the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary into the systemic circulation, which in turn causes increased gonadal activity.

the Greek word *tanus*, meaning 'stretched' or 'elongated')[5], and their long, thick processes encircle the capillaries and neuronal termini, forming a physical barrier between them[6]. But the structural association of tanycytes with nerve fibres and capillaries is changeable, and so allows modulation of the concentrations of neurohormones released into the capillaries. The capillaries then feed into the portal vessels, and the neurohormones are conveyed to the secondary capillary plexus in the pars distalis, which is the main unit of the anterior pituitary. Here, they trigger the secretion of pituitary hormones to regulate various endocrine organs in the body. This canonical view of the hypothalamic–pituitary system has no role for the pars tuberalis.

The pars tuberalis is composed of small, specialized glandular cells that are different from the endocrine cells of the pars distalis[7]. Significantly, in most species it is located next to the median eminence. The two structures face each other and are separated by a dense capillary plexus that connects to the portal vessels. By means of these local vascular connections, thyrotropin, released from the pars tuberalis cells in response to increased day length, enters the capillary plexus of the median eminence and binds to thyrotropin receptors on the tanycytes encircling the capillaries. Nakao *et al.*[2] find that, once activated, tanycytes induce the expression of various gene transcription factors and enzymes, and undergo conformational changes that allow increased GnRH release into the capillaries. These findings strongly suggest that the pars tuberalis and median eminence form a functional and structural unit.

What are the implications of Nakao and colleagues' findings[2] for the photoperiodic response in mammals? Although the route taken by photoperiodic signals to reach the median eminence–pars tuberalis functional unit is different in birds and mammals, the authors' observations in Japanese quail are likely to hold for mammals too: similar, if not identical, photoperiod-dependent changes in gene expression have been reported in the median eminence and pars tuberalis of the hamster brain[8]. Another implication of Nakao and colleagues' findings is that the median eminence–pars tuberalis complex is the key site responsible for the spontaneous restoration of gonadal activity that occurs after an animal is kept under a photoperiodic condition called photorefractoriness[9]. The incorporation of photorefractoriness into photoperiodic responses (photoperiodicity) gives seasonal breeding animals, whether birds or mammals, greater flexibility in adjusting the length and time of their breeding season.

The transformation of a light signal into endocrine signals is a feature that is shared by the circadian rhythm and photoperiodicity. In the circadian system this transformation is used to entrain circadian clocks throughout the body; for example, light stimulates adrenal glands to secrete glucocorticoid hormones into the systemic circulation[10]. In photoperiodicity, this conversion occurs at the junction of the hypothalamus and the pituitary, as the main target of the photoperiodic signal is the hypothalamic–pituitary–gonadal hormonal axis. There, the light signal induces the release of thyrotropin from pars tuberalis cells, which stimulates tanycytes to 'draw' GnRH into the portal circulation. So, in photoperiodicity, thyrotropin works locally as a tanycyte-stimulating hormone. ■

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50 YEARS AGO

'Interpretation of science to the public'. By Sir Lawrence Bragg — It is perennially surprising to find that it is the simpler experiments which have the warmest reception, especially if they can be done on a spectacular scale. The audience likes to see 'the works'. It is of little help to do something mysterious in a box which makes a pointer move over a scale. The simple experiment is effective because it can be grasped completely, and the members of the audience have the pleasurable feeling that it has become a part of their own experience ... The other guiding principle of the popular lecture is that of starting with something with which the audience is thoroughly familiar in every-day life, and leading them further with that as a basis ... [The lecturer] has to put himself in the place of the intelligent layman and realize that ideas and experiences so familiar to him are unexplored country to his listener.
From *Nature* 22 March 1958.

100 YEARS AGO

Lest some readers should infer from your obituary note on Sir Denzil Ibbetson (March 12, p. 443) that this distinguished anthropologist invented the word "godlings" for the rural deities of India, it is worth noting that "godling" was good English in the sixteenth century, and has never been allowed to drop. The Philological Society's "New English Dictionary" quotes Lambarde's "Perambulation of Kent" (1570–6) on raising altars "to this our newe found Godlyng" ... Coleridge preferred "godkin" for a minor deity with masculine attributes, but sanctioned "goddessling". Charles Colton in 1675 permitted a certain cult of "little Goddikins"; Coventry Patmore regarded "godlet" as the more dignified appellative. Anthropologists have therefore had a fairly ample choice; but it should be added that in some of the above examples, at least, Dr. Murray and his coadjutors suspected a "jocular" intention.
From *Nature* 19 March 1908.

50 & 100 YEARS AGO

EXTRASOLAR PLANETS

A whiff of methane

Adam P. Showman

Investigations of planets outside our Solar System are becoming ever more sophisticated. The latest development is the discovery of a carbon-containing molecule in the atmosphere of one such extrasolar body.

Methane is a constituent of many of the atmospheres in our Solar System: those of Earth, Mars, Titan and the gas giants Jupiter, Saturn, Uranus and Neptune all contain traces of it. Despite its low abundance, the methane provides telling clues about planetary formation, evolution, weather, photochemistry and — in the case of Earth — life. We have discovered more than 270 planets outside our Solar System, but for most of them we know nothing more than their mass and orbital properties. Owing to their immense distances from us, and their feeble brightness relative to the incandescence of the stars they orbit, observationally inferring anything about their composition is extremely difficult.

That, however, is just what Swain *et al.*[1] have achieved. On page 329 of this issue, they present the first detection of methane, CH_4, on an extrasolar planet. They also confirm a previous detection[2] of water vapour in the atmosphere of this planet, called HD 189733b, and provide a more robust estimate of its abundance. The planet is a 'hot Jupiter' that orbits only 0.03 Earth–Sun distances from its star. Blasted by starlight, the planet's atmospheric temperatures reach a searing 1,000 K.

Swain and colleagues' finding is the first detection of any carbon-bearing molecule on a planet outside our Solar System. It was made possible by the fact that HD 189733b is a transiting planet — one whose orbit is fortuitously aligned such that the planet periodically passes in front of its star as viewed from Earth. Such transits are relatively easy to detect, even using small telescopes on Earth[3]. In the case of HD 189733b, the planetary transit blocks more than 2% of the starlight, allowing a direct estimate of the planet's radius. Much harder to detect are the subtle variations of this absorption with wavelength that yield clues to atmospheric composition. At wavelengths at which the atmosphere is transparent, starlight passes through the atmosphere unimpeded. At wavelengths that are more opaque, the atmosphere blocks the starlight and the total absorption seen from Earth is greater (Fig. 1). In this way, Swain *et al.*[1] used observations from the NICMOS camera on the Hubble Space Telescope to construct an infrared spectrum of the planet as seen in transmitted starlight; that spectrum shows the tell-tale absorption features of methane and water vapour in the planet's atmosphere.

So what does the measurement tell us about planetary behaviour? The methane abundances on Jupiter, Saturn and both Uranus and Neptune correspond to respective carbon/hydrogen ratios of 3, 7 and ~30–40 times the C/H ratio in the Sun's atmosphere (where carbon resides entirely in atomic form). This provides important clues about planetary formation, because it suggests that these planets not only received carbon from the gas of the solar nebula (which presumably had nearly the solar C/H ratio), but that they also absorbed huge quantities of carbonaceous rocky and icy material as they were forming. For extrasolar planets, one thus expects the C/H ratio (as well as the ratio of other heavy elements to hydrogen) to provide a crucial probe of how much solid material accreted with the gas.

But the story is more complicated for HD 189733b. The observationally inferred

DRUG DISCOVERY

Schistosome treatment

The statistics are grim. Schistosomiasis — a tropical disease caused by flatworms of the genus *Schistosoma* — kills 280,000 people annually. Moreover, 200 million people are infected with the worm, and a further 800 million or so are at risk. An effective, low-cost drug called praziquantel is available. But it is heavily and regularly prescribed and, as there is currently no vaccine or alternative drug available, the danger of schistosome parasites evolving resistance to the drug is looming. So the discovery by Ahmed Sayed and colleagues, at Illinois State University in Normal and the US National Institutes of Health (NIH) in Bethesda, Maryland, of a drug that may be as effective as praziquantel, if not more so, is good news (A. A. Sayed *et al. Nature Med.* doi:10.1038/nm1737; 2008).

Within their human host, schistosome parasites reside in aerobic environments — skin, blood, lungs and liver. This means they must be able to avoid damage caused by reactive oxygen species. The authors therefore took advantage of a valuable resource — the NIH Chemical Genomics Center chemical repository — to screen thousands of compounds for their ability to target the antioxidant pathway in one of the most widespread of schistosome species, *Schistosoma mansoni* (pictured).

The screen led to the identification of two groups of compounds, phosphinic amides and oxadiazole 2-oxides, which inhibit an enzyme known as TGR — a multifunctional agent that is uniquely responsible for the detoxification of reactive oxygen species in schistosome parasites. One oxadiazole called furoxan was particularly noteworthy for being highly potent at low doses.

Furoxan did well in subsequent tests. The authors found that, within 24 hours, drug concentrations of just 10 micromolar killed 100% of adult *S. mansoni* grown in culture, and concentrations of 2 micromolar had the same effect over 5 days.

D. SCHARF/SPL

Moreover, furoxan was active against two other worm species that also cause schistosomiasis.

Human white blood cells produce nitric oxide, which can kill larval schistosomes. Sayed and colleagues show that, as well as inhibiting TGR activity, furoxan reacts with TGR to produce nitric oxide, increasing the compound's toxic effect on the parasite.

The authors also tested the toxicity of furoxan in mouse cell lines and found that its toxicity is not very different from that of praziquantel. Investigating the efficacy of furoxan in overcoming schistosome infection in mice revealed that, depending on the stage in the life-cycle of *S. mansoni* at which the drug was administered, the disease burden was reduced by at least 88%. Such efficacy exceeds the criteria set by the World Health Organization for potential lead compounds for the treatment of schistosomiasis. Furoxan is thus a highly promising compound for use as an alternative or supplement to praziquantel — the two drugs function through different mechanisms. Efforts are already under way to generate furoxan derivatives suitable for use in humans.

Sadaf Shadan

methane abundance[1] corresponds to a mole fraction (that is, a ratio of methane to background hydrogen-rich gas) of only 5×10^{-5} or less, which corresponds to only 10% or less of the C/H ratio of the parent star. So where is all the carbon? Methane becomes thermodynamically disfavoured as the temperature rises above 1,000 K; under such conditions, carbon prefers to combine with oxygen to form carbon monoxide (CO) instead. Because the temperature of HD 189733b lies near this transition point, early predictions[4] suggested that the dominant carbon carrier would be CO but that detectable quantities of methane would also exist. The discovery of methane at abundances much less than solar thus makes sense theoretically. Consistent with these ideas, models[5] of an infrared emission spectrum of HD 189733b, recently produced with data gathered by the Spitzer Space Telescope[6], suggest the indirect signature of CO.

Interestingly, Swain *et al.*[1] and Tinetti *et al.*[2] infer a water-vapour mole fraction of 5×10^{-4} for HD 189733b. This value has important implications for the planet's O/H and C/H ratios, and hence provides constraints on planetary formation and evolution. How so? For solar elemental ratios — thought to be similar to abundances in the planet's host star — about one oxygen atom is available per 10^3 hydrogen molecules. Likewise, about half a carbon atom is available per 10^3 hydrogen molecules. If carbon resides primarily in CO, as expected for HD 189733b, then the CO locks up half the oxygen atoms, leaving the other half to form water and implying a water mole fraction of about 5×10^{-4}. According to this chain of logic, the inferred water abundance on HD 189733b implies C/H and O/H ratios that are consistent with the values in our Sun and, potentially, in the planet's star. Although lack of knowledge of the C/O ratio on HD 189733b prevents a definitive assessment, these constraints hint that — unlike the giant planets in our Solar System — HD 189733b is not substantially enriched in heavy elements (such as carbon and oxygen) relative to its parent star.

The methane abundance could also hold clues to the exotic weather on this hot Jupiter. If the atmospheric composition is in chemical equilibrium, the carbon on the hot dayside should reside almost entirely in CO, whereas methane would be important on the colder night-side. A day–night map of temperatures on this planet[7] suggests the existence of fast winds that can rapidly homogenize the temperature. Because of the finite time needed to interconvert between methane and CO, the methane and CO abundances (and their spatial variation) surely contain information about the atmospheric temperatures and transport timescales[8]. Additional observations and models will be needed to extract this information.

These are exciting times for studies of extrasolar planets. The past few years have seen an avalanche of unprecedented observations constraining the physical properties of HD 189733b and other transiting hot Jupiters.

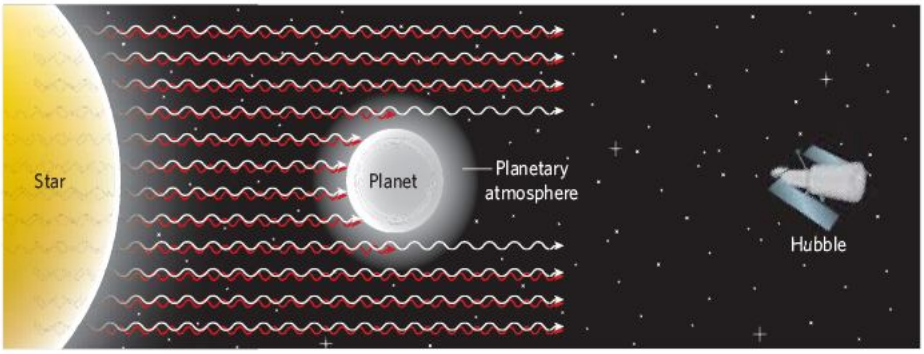

Figure 1 | Methane detection. When a planet passes in front of its star as viewed from Earth, the planetary atmosphere preferentially blocks more of the starlight at wavelengths where the atmosphere is opaque (red) and less at wavelengths where the atmosphere is transparent (white). In this way, Swain *et al.*[1] used the Hubble Space Telescope to obtain a transmission spectrum of the hot Jupiter HD 189733b, which reveals the presence of methane in the planet's atmosphere and confirms the presence of water vapour.

Thirteen years after the discovery of the first extrasolar planet around a Sun-like star, we are finally moving beyond simply discovering such planets to truly characterizing them as worlds. Although the big guns in these discoveries — the Hubble and Spitzer space telescopes — are nearing old age, next-generation platforms such as NASA's James Webb Space Telescope are under development. We are thus now seeing but the opening salvo in a revolution that will extend humankind's view of planetary worlds far beyond the provincial boundaries of our Solar System. ■

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HUMAN BEHAVIOUR

Punisher pays

Manfred Milinski and Bettina Rockenbach

The tendency of humans to punish perceived free-loaders, even at a cost to themselves, is an evolutionary puzzle: punishers perish, and those who benefit the most are those who have never punished at all.

Humans are champions of cooperation. Reciprocity — the idea that, if I help you this time, you'll help me next time[1] — is a secret of our success. But how do I avoid being the sucker when someone I've helped refuses to pay me back? Social-dilemma games, which in the laboratory mimic human social interactions, have shown that the opportunity to punish is an effective curb on 'defectors', even when punishment not only hurts the punished, but also the punisher[2–5]. We see that kind of behaviour outside the laboratory too: bystanders suffer personal injury intervening in altercations; environmental activists risk their lives fighting destructive acts; and so on.

On page 348 of this issue, Dreber *et al.*[6] quantify who profits from this 'costly punishment'. Their findings are intriguing. Although costly punishment induces cooperation, its cost destroys all gains from increased cooperation, not just for the punisher, but for the whole group. At the end of the game, those who punished were the ultimate losers; the absolute winners had never punished. Explaining why costly punishment is used at all, if not even the group seems to benefit, becomes even more of a challenge.

The authors used a variant of the classic two-person 'prisoner's dilemma' game, in which players have a binary choice of cooperation or defection. If I cooperate with you, I lose one unit of money so that you gain two; if I defect, I gain one unit and you lose one. That way, if we both cooperate, each of us has a net gain of one unit. If we both defect, neither of us gains anything; so cooperation pays. But if you cooperate and I defect, I gain three units and you lose two. That makes defection tempting for most people, and cooperation generally breaks down at some point in a prisoner's dilemma game.

POPPERFOTO/GETTY IMAGES

Figure 1 | Winners don't punish. Mahatma Gandhi is a prime example of the maxim that Dreber *et al.* establish in their social-dilemma games[6]: that those who do not punish come out on top in societal interactions.

A strategy that emerges is 'tit-for-tat'[7]: players begin cooperatively, and then copy their partner's last move, cooperating with cooperators and defecting with defectors — thus avoiding being the sucker.

In Dreber and colleagues' extension of this game[6], participants could choose from three options in each round: cooperate, defect or punish. Punishment here means losing one money unit so that the other player loses four. There are thus two ways of expressing disapproval: the moderate way of defection, and the harsh way of costly punishment. Subjects made use of the harsher option in 7% of all choices. A single punishment act rarely re-established cooperation; indeed, it often led to mutual back-stabbing. But overall, cooperation increased from 21% in the prisoner's dilemma game, used by the authors as a control, to 52%, although the tit-for-tat strategy was an option in either case.

A success story, one might think. Not for the punishers: the more a player had used the punishment option, the lower that individual's final profit was. The final, aggregated pay-off of all participants (quantifying the benefit to society as a whole) was the same in the games with and without the punishment option.

If both punishers and the punished lose through punishment, someone must have profited. Indeed: cooperators who did not punish at all gained even more in the games where punishment was possible than the best-performing participants in the control. Thus, it would seem, winners don't punish; and punishers perish (Fig. 1).

Dreber *et al.* conclude that costly punishment is a 'maladaptive' behaviour in social-dilemma situations — one that is fundamentally counterproductive, because it pays off neither for the punisher nor for the group. Thus, although it frequently induces cooperation, it can't have evolved for inducing cooperation. Not even the cooperation-enhancing effect appears consistently in social-dilemma games. In some societies, not only free-loaders but also high contributors are punished, which dampens and sometimes even removes the cooperation-enhancing effect of punishment[8].

Dreber *et al.* argue that punishment has evolved for another purpose, such as coercing individuals into submission, or establishing dominance hierarchies. But the fact remains that, given the choice, players of social-dilemma games have been shown to prefer an environment where punishment is possible. That preference pays off when participants, punishers as well as non-punishers, enter this environment after the initial period of high punishment is over and cooperation dominates[4].

If costly punishment is detrimental to personal evolutionary fitness in a certain situation, we should have evolved the ability to suppress it in that context. Evidence that we have comes from ultimatum games, in which one player decides how to split a sum of money, and the second player can either accept the offer (in which case both players receive the proposed share) or reject it (in which case neither player wins anything). Neurological tests have shown that humans have a stronger activation of brain areas related to both emotion and cognition when unfair offers in an ultimatum game come from other humans than when the same offers, and monetary consequences, come from a computer[9]. Similarly, in experiments where subjects could choose between costly punishment of the free-loaders and helping cooperative players to gain, costly punishment was reduced to a third; the few remaining punishing acts were concentrated on the worst defectors[10]. In our view, this ability to reduce the use of costly punishment makes it unlikely that it is just an unavoidable by-product of something else, such as an inability to control anger.

To provide punishers with an overall net benefit, costly punishment must be greatly rewarded in another context. Perhaps punishers gain a special kind of reputation that is advantageous elsewhere. But so far, there has been no conclusive evidence for such a delayed pay-off, and so costly punishment remains one of the most thorny puzzles in human social dilemmas. Dreber and colleagues' results make it plain that we are still a long way from understanding the dark side of human sociality. ■

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QUANTUM PHYSICS

Disturbance without the force

Akira Tonomura and Franco Nori

Charged particles influenced by electromagnetic fields, even when the two never touch? Surely, it can only be quantum physics. But surprisingly, the quantum nature of this particular effect has been disputed.

In the phenomenon known as the Aharonov–Bohm effect, magnetic forces seem to act on charged particles such as electrons — even though the particles do not cross any magnetic field lines. Is this evidence for electromagnetic forces that work in new and unsuspected ways? Or is it just that infamous source of Albert Einstein's discomfort — quantum-mechanical 'spooky action at a distance'? In the latest chapter in an involved history, detailed in *Physical Review Letters*, Caprez *et al.*[1] provide convincing evidence for the second of these options: that the Aharonov–Bohm effect is purely quantum mechanical in origin.

The history stretches back into the mid-nineteenth century, when Michael Faraday first proposed that lines of electric and magnetic force extend out into the empty space surrounding both magnets and electrical charges. The idea initially received a cool reception — ironically, in view of later developments, because Faraday's peers were wedded to the idea that these forces acted at a distance. But field lines, whose density gives the 'flux

density', the magnitude of the field at a point, have proved both useful and exceptionally durable. A little later, Faraday's concept of electric and magnetic fields was fleshed out mathematically by two other titans of nineteenth-century physics, William Thomson (Lord Kelvin) and James Clerk Maxwell, who introduced and developed the unifying concept of the vector potential. In Maxwell's eponymous equations, in which he laid out his unified theory of electromagnetism, an electric field is produced when this vector potential changes with time; a magnetic field is produced when the vector potential changes spatially and has a vortex.

The vector potential, although initially regarded as a physical quantity in this formulation, became, in later standard treatments by Heinrich Hertz and Oliver Heaviside, a mere mathematical auxiliary: convenient for calculations, but possessing no direct physical meaning. Almost a century passed before, in 1959, Yakir Aharonov and David Bohm proposed[2] that in certain experimental contexts the vector potential itself would indeed have measurable effects, and thus its own physical reality. A paradigm had begun to shift: in modern physics, vector potentials, in the guise of 'gauge fields', are regarded as the most fundamental physical quantity in the quantum theories of the fundamental forces[3–6].

In the quantum world, particles such as electrons can behave as waves. Electric and magnetic fields can shift electrons' wave fronts (or their phases) much as obstacles disturb ripples on a water surface. What Aharonov and Bohm concluded[2], however, was remarkable: that the phases of electrons passing through regions entirely free from electromagnetic fields could also be shifted. They envisaged an experiment in which two electron beams pass by on either side of an infinitely long, perfectly shielded magnetic coil, such that the electrons never directly experience magnetic fields or forces (Fig. 1). Aharonov and Bohm calculated that, when these two beams are subsequently guided to overlap and form interference fringes, the phases of the two beams would be shifted relative to one another. They attributed this effect to the vector potential, which does not vanish in the regions around the magnet through which the electrons had passed.

The Aharonov–Bohm effect is pivotal: it is directly related to fundamental problems in quantum mechanics, such as whether a wavefunction has a single value at a point in space and the quantization of magnetic flux. But it is also controversial, in part because of the modern philosophical aversion, expressed by Einstein and others, to the concept of action at a distance. Conclusive evidence for its existence[7] was obtained only in 1986 by using, instead of the infinitely long magnets of the theoretical formulation, doughnut-shaped (toroidal) magnets covered with superconductors to shield any leakage of magnetic flux.

Even though the Aharonov–Bohm effect is regarded as a consequence of the Schrödinger equation — the general equation governing the evolution of a quantum system — questions have been raised as to whether it is a purely quantum-mechanical phenomenon or not[3,4,8,9]. Several people have attempted to interpret the Aharonov–Bohm effect in the context of a classical interaction between the incident electrons and the coil[3]. For example, Timothy Boyer[9] has postulated a 'lag effect' ascribed to a force applied to the electrons. In standard classical theory, electric and magnetic fields are defined as forces exerted on charged particles, and so no forces would be exerted on electrons passing on either side of Aharonov and Bohm's perfectly shielded magnetic coil[3]. Where the necessary force, which would have to accelerate the electrons on one side of the coil and decelerate them on the other, would come from is unclear.

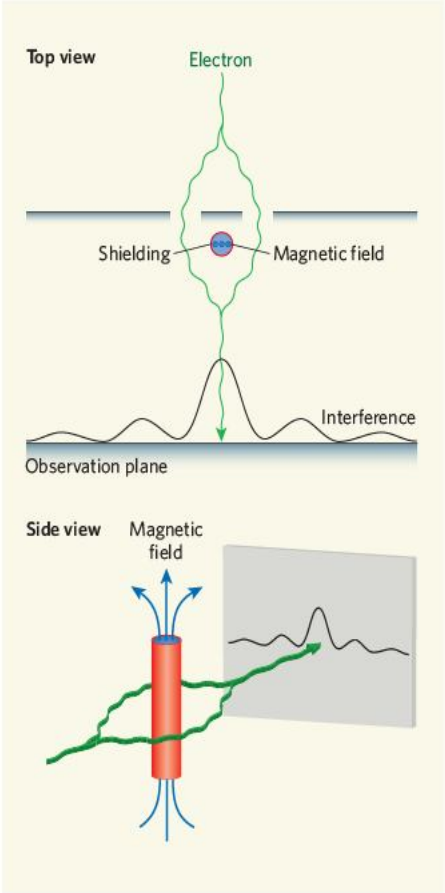

Figure 1 | The Aharonov–Bohm effect. In Aharonov and Bohm's original theoretical formulation of their effect, an electron beam is split into two, passing on either side of an (infinitely) long, perfectly shielded magnet. The result is a phase-shift evident in an interference pattern formed when the electron beams are recombined. It seems that the electrons 'feel' the non-local presence of the magnetic field through its associated vector potential, which permeates the space around the coil. An analogous effect, the Aharonov–Casher effect, which applies to 'quantum magnetic dipoles' (spins), can be demonstrated by replacing the magnet by an electrically charged cylinder. Caprez and colleagues' experiments[1] with a pulsed electron beam passed through a toroidal magnet seem to confirm that no unknown forces are involved in the Aharonov–Bohm effect — it is a purely quantum-mechanical phenomenon.

The contribution of Caprez *et al.*[1] is to rule out the possibility that the Aharonov–Bohm effect can be explained through the existence of such forces. They do this by timing how long the electrons of a pulsed beam, created by shaving electrons off a nanoscale tip using a femtosecond laser beam, take to pass through field-free regions in the hole of a toroidal magnet to a detector. Crucially, even when the electric current flowing through the magnet was changed — which would be expected to affect the magnitude of any possible unknown force — no additional time delay of electrons was detected. Thus, the Aharonov–Bohm effect would seem to be confirmed as a purely quantum-mechanical effect. Action at a distance is alive and well.

This use of a pulsed electron beam[10] is innovative and worthy of attention, as one can envisage its use in experiments to probe other, counterintuitive quantum effects. For example, it would be intriguing to test with this new technique the 'electrical' Aharonov–Bohm effect[3], in which two electron beams passing through two long, shielded metal cylinders, and experiencing no forces, can be phase-shifted when a potential is applied to one of the cylinders. To test this effect, both a pulsed electron beam and the synchronous application of the potential to the cylinder only when the beams are well inside are indispensable.

Faraday's magnetic field lines were essential to give us a mental picture of how the forces of classical electromagnetism worked, a picture that proved crucial to the development of the first electromechanical devices in the late nineteenth century. In much the same way, the outcome of these fundamental experiments might give us more of a handle on mysterious quantum effects such as action at a distance — and how we might use them to our advantage. ■

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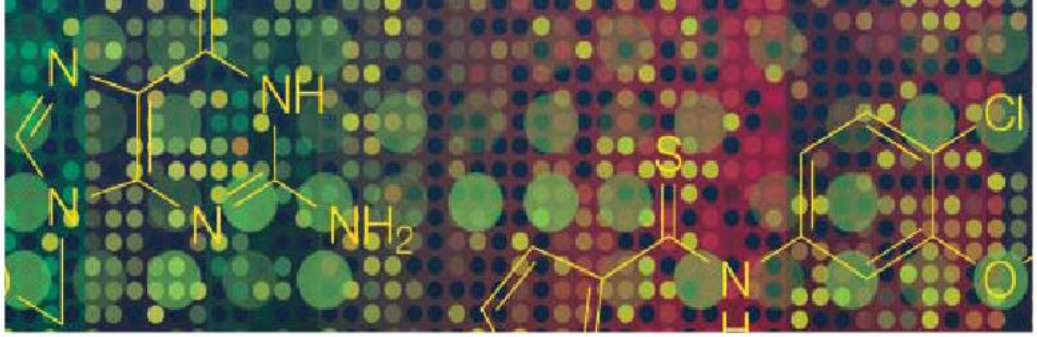

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REVIEWS

Science and technology for water purification in the coming decades

Mark A. Shannon[1,4], Paul W. Bohn[1,2], Menachem Elimelech[1,3], John G. Georgiadis[1,4], Benito J. Mariñas[1,5] & Anne M. Mayes[1,6]

One of the most pervasive problems afflicting people throughout the world is inadequate access to clean water and sanitation. Problems with water are expected to grow worse in the coming decades, with water scarcity occurring globally, even in regions currently considered water-rich. Addressing these problems calls out for a tremendous amount of research to be conducted to identify robust new methods of purifying water at lower cost and with less energy, while at the same time minimizing the use of chemicals and impact on the environment. Here we highlight some of the science and technology being developed to improve the disinfection and decontamination of water, as well as efforts to increase water supplies through the safe re-use of wastewater and efficient desalination of sea and brackish water.

The many problems worldwide associated with the lack of clean, fresh water are well known: 1.2 billion people lack access to safe drinking water, 2.6 billion have little or no sanitation, millions of people die annually—3,900 children a day—from diseases transmitted through unsafe water or human excreta[1]. Countless more are sickened from disease and contamination. Intestinal parasitic infections and diarrheal diseases caused by waterborne bacteria and enteric viruses have become a leading cause of malnutrition owing to poor digestion of the food eaten by people sickened by water[2,3]. In both developing and industrialized nations, a growing number of contaminants are entering water supplies from human activity: from traditional compounds such as heavy metals and distillates to emerging micropollutants such as endocrine disrupters and nitrosoamines. Increasingly, public health and environmental concerns drive efforts to decontaminate waters previously considered clean. More effective, lower-cost, robust methods to disinfect and decontaminate waters from source to point-of-use are needed, without further stressing the environment or endangering human health by the treatment itself.

Water also strongly affects energy and food production, industrial output, and the quality of our environment, affecting the economies of both developing and industrialized nations. Many freshwater aquifers are being contaminated and overdrawn in populous regions—some irreversibly—or suffer saltwater intrusion along coastal regions. With agriculture, livestock and energy consuming more than 80% of all water for human use, demand for fresh water is expected to increase with population growth, further stressing traditional sources. The shift to biofuels for energy may add further demands for irrigation and refining. Alarmingly, within 30 years receding glaciers may cause major rivers such as the Brahmaputra, Ganges, Yellow (which already at times no longer runs to the sea) and Mekong rivers, which serve China, India and Southeast Asia, to become intermittent, imperilling over 1.5 billion people during the dry months[4,5]. Even industrialized nations in North America and Europe, and those in Andean countries in South America, could see major disruptions to agriculture, hydroelectric and thermoelectric generation, and municipal water supplies from reductions in snowmelt and/or loss of glaciers[6,7]. In the coming decades, water scarcity may be a watchword that prompts action ranging from wholesale population migration to war, unless new ways to supply clean water are found.

Fortunately, a recent flurry of activity in water treatment research offers hope in mitigating the impact of impaired waters around the world. Conventional methods of water disinfection, decontamination and desalination can address many of these problems with quality and supply. However, these treatment methods are often chemically, energetically and operationally intensive, focused on large systems, and thus require considerable infusion of capital, engineering expertise and infrastructure, all of which precludes their use in much of the world. Even in highly industrialized countries, the costs and time needed to develop state-of-the-art conventional water and wastewater treatment facilities make it arduous to address all the problems. Furthermore, intensive chemical treatments (such as those involving ammonia, chlorine compounds, hydrochloric acid, sodium hydroxide, ozone, permanganate, alum and ferric salts, coagulation and filtration aids, anti-scalants, corrosion control chemicals, and ion exchange resins and regenerants) and residuals resulting from treatment (sludge, brines, toxic waste) can add to the problems of contamination and salting of freshwater sources. Moreover, chemically intensive treatment methods in many regions of the world cannot be used because of the lack of appropriate infrastructure.

However, even within central Europe there has been a movement towards reducing chemical treatment via engineered 'natural' systems for drinking-water production in order to reduce residual chemicals in the distribution systems[8]. Fortunately there is much more that science and technology can do to mitigate environmental impact and increase efficiency because current treatment methods are still far from natural-law limits in their ability to separate compounds, deactivate or remove deleterious pathogens and chemical agents, transport water molecules, and move ions against concentration gradients. Our expectation is that by focusing on the science of the aqueous interface between constituents in water and the materials used for treatment, new, sustainable, affordable, safe and robust

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methods to increase supplies and purify water can be developed and implemented to serve people throughout the world.

Here, we highlight some of the science and next-generation systems being pursued: to disinfect water, removing current and emerging pathogens without intensive use of chemicals or production of toxic byproducts; to sense, transform, and remove low-concentration contaminants in high backgrounds of potable constituents at lower cost; and to re-use wastewater and desalinate water from sea and inland saline aquifers, all of which hold great promise for effectively increasing water supplies. To realize these challenging goals, many open research questions need to be addressed. Our thesis is that research will enable improved disinfection, decontamination, re-use and desalination methods to work in concert to improve health, safeguard the environment, and reduce water scarcity, not just in the industrialized world, but in the developing world, where less chemical- and energy-intensive technologies are greatly needed.

Disinfection

An overarching goal for providing safe water is affordably and robustly to disinfect water from traditional and emerging pathogens, without creating more problems due to the disinfection process itself. Waterborne pathogens have a devastating effect on public health, especially in the developing countries of sub-Saharan Africa and southeast Asia[9]. Waterborne infectious agents responsible for these diseases include a variety of helminthes, protozoa, fungi, bacteria, rickettsiae, viruses and prions[10]. While some infectious agents have been eradicated or diminished, new ones continue to emerge and so disinfecting water has become increasingly more challenging. Viruses are of particular concern, accounting, together with prions, for nearly half of all emerging pathogens in the last two to three decades[9]. Enteric viruses received less attention in the past compared with bacterial pathogens (for example, *Vibrio cholerae*) and protozoan parasites (for example, *Cryptosporidium parvum*), partly because they were difficult to detect, and partly because free chlorine (the main disinfectant used worldwide because of its potency and low cost) was very effective in inactivating them. However, free chlorine is ineffective in controlling waterborne pathogens such as *C. parvum* and *Mycobacterium avium*. *M. avium* in particular is ubiquitous in biofilms within water distribution systems around the world, with remarkable resistance to chlorine at the high pH and low temperature of natural water. Indeed, ageing and deterioration of drinking water distribution systems and the associated growing of biofilms within them has emerged as a key infrastructure rehabilitation challenge: significant resources are needed to maintain and upgrade distribution systems. In the USA, where large numbers of such old systems exist, disinfectants are required to suppress pathogens within the system. Halogenated disinfection strategies for treatment and distribution systems produce toxic disinfection by-products (DBPs) such as trihalomethanes and haloacetic acids. Recent US disinfection regulations[11,12] require the control of *C. parvum* oocysts while minimizing the formation of certain DBPs, which might force some drinking-water utilities to discard free chlorine disinfection and implement alternative technologies.

Therefore, the effective control of waterborne pathogens in drinking water calls for the development of new disinfection strategies, including multiple-barrier approaches that provide reliable physicochemical removal (for example, coagulation, flocculation, sedimentation, and media or membrane filtration) along with effective photon-based and/or chemical inactivation. The 1993 outbreak of cryptosporidiosis in Milwaukee, Wisconsin, USA, in which approximately 400,000 people were infected and more than 100 died, was a wake-up call for the US drinking-water industry. They were reminded that relying exclusively on physicochemical removal, which can suffer from malfunctions arising from defects in manufacturing or operation, can have a devastating effect on public health.

The use of light from visible to ultraviolet (UV) to photochemically inactivate pathogens has recently seen a resurgence in interest, notwithstanding the historical use of sunlight to disinfect water. Sequential disinfection schemes such as UV/combined chlorine and ozone/combined chlorine are being considered by many drinking-water utilities as the inactivation component of their multiple-barrier treatment plants because, compared with free chlorine, both UV and ozone are very effective in controlling *C. parvum* oocysts. In addition, combined chlorine can provide a residual in distribution systems without forming high levels of regulated DBPs. However, changing disinfection technologies has raised new concerns because viruses, although effectively controlled by ozone, are resistant to both UV and combined chlorine disinfection. Moreover, ozone can form the DBP carcinogen bromate ion in water containing bromide ions, and combined chlorine can form other unregulated DBPs, for example, haloacetonitriles and iodoacetic acid[13,14], that may be more toxic and carcinogenic than those associated with free chlorine.

The situation in developing countries is similar. International agencies and non-governmental organizations have introduced the use of sunlight irradiation of water within PET (polyethylene terephthalate) bottles to kill pathogens, and are promoting the use of sodium hypochlorite for point-of-use disinfection of drinking water in rural areas (for example, the CDC SafeWater System)[15]. Although these initiatives have lowered the incidence of gastrointestinal disease, owing to the lack of adequate sanitation, the source waters in these areas contain ammonia and organic nitrogen that react with the sodium hypochlorite to form combined chlorine species that are ineffective in inactivating viruses. Furthermore, relatively high levels of toxic DBPs can form in the presence of high concentrations of organic matter associated with inadequate sanitation.

To develop alternatives to chlorine (free and combined) and UV disinfection for the control of waterborne viruses requires significant advances in understanding how viruses are inactivated by the benchmark (chlorine and UV) methods and by any new technologies. The goal is to match or improve on the positive aspects of chlorine and UV disinfection while avoiding the negative effects. To do so requires several questions to be answered. It is well established that both UV light and the free chlorine species hypochlorous acid (HOCl) react with various amino acids in the virus capsid proteins as well as with the nucleic acid protected by the capsid[16,17]. However, the actual limiting step (that is, the molecular target and its level of damage) responsible for inactivation is not yet known. Developing a process that targets that inactivation mechanism may create a new, safe, and robust disinfection method.

For example, many species of adenovirus—the waterborne pathogens with highest resistance to UV inactivation—use their fibre head (Fig. 1a) to attach to the amino-terminal D1 domain of the coxsackievirus-and-adenovirus receptor (CAR) of host cells[18]. Amino acid sequence alignments have shown that the hydrophobic side group of tyrosine and ionizable basic side groups of histidine and lysine in the fibre head associate with CAR amino acids (Fig. 1b) and thus play a role in the attachment of adenovirus to the host cell[19]. Consequently, oxidization of the phenolic group of tyrosine, and formation of reactive chloramines with the amino groups of histidine and lysine[20–23] could contribute to changing the conformation of the adenovirus head protein and inhibiting binding to receptors, thus effectively inactivating the virion. HOCl also reacts with nucleic acid and amino acid residues involved in many steps of the infection cycle of viruses, such as cell entry (endocytosis and endosomal lysis), intracellular trafficking and nuclear delivery, in the case of adenovirus[24]. Thus, even if the virus penetrates the cell, the infection cycle could be inhibited at some subsequent step. A potential problem with this strategy is that once inside the cell, the virion might manipulate the host cell to repair the damage and subsequently complete the infection cycle[25]. Consequently, disinfection processes that target the proteins responsible for attachment and penetration would avoid the unwanted possibility of genome repair.

A new generation of disinfection processes to control viruses should be capable of selective reactions with the key residues in

proteins responsible for binding to host cell receptor molecules. Heterogeneous processes are envisioned that would use complementary nanostructured and functionalized surfaces that mimic the structure and functionality of the receptors of target protein residues. These structures should have both high affinity and specificity and be relatively inert to the large amounts of organic matter ubiquitous in natural water. The surfaces of new materials could be designed with arrays of sites that serve to trap all waterborne viral pathogens via binding to host receptors.

A futuristic disinfection method involves the combined use of photons and engineered nanostructures. Although UV is effective for inactivating waterborne bacteria and protozoa cysts and oocysts, it is not very effective for viral pathogens. However, UV light is capable of activating photocatalytic materials such as titania (TiO_2), which are capable of inactivating viruses. Furthermore, new photocatalysts such as TiO_2 doped with nitrogen (TiON), or co-doped with nitrogen and a metal such as palladium, can be activated with visible light[26] (which could potentially inactivate viruses and other waterborne pathogens with much lower energy use than UV), or even with sunlight (for deployment anywhere with bright sunlight). Of particular interest are materials and systems that use low-cost visible lamp light and sunlight to achieve sufficiently high throughput. Low throughput rates have thus far limited adoption of photoinactivation. Throughput rates depend on factors such as incident light flux and wavelength, absorption length through water, geometry, reactor hydrodynamics, contact efficiency of species in water on the photocatalysts and, critically, the inactivation kinetics. Moreover, we need to improve our understanding of the mechanisms for the interactions of pathogens, in particular virions, with excited photocatalyst surfaces and adherent active moieties, such as hydroxyl radicals and superoxides. The physicochemical structure of such surfaces would need to be optimized for maximum selective affinity of target viral capsid molecular motifs.

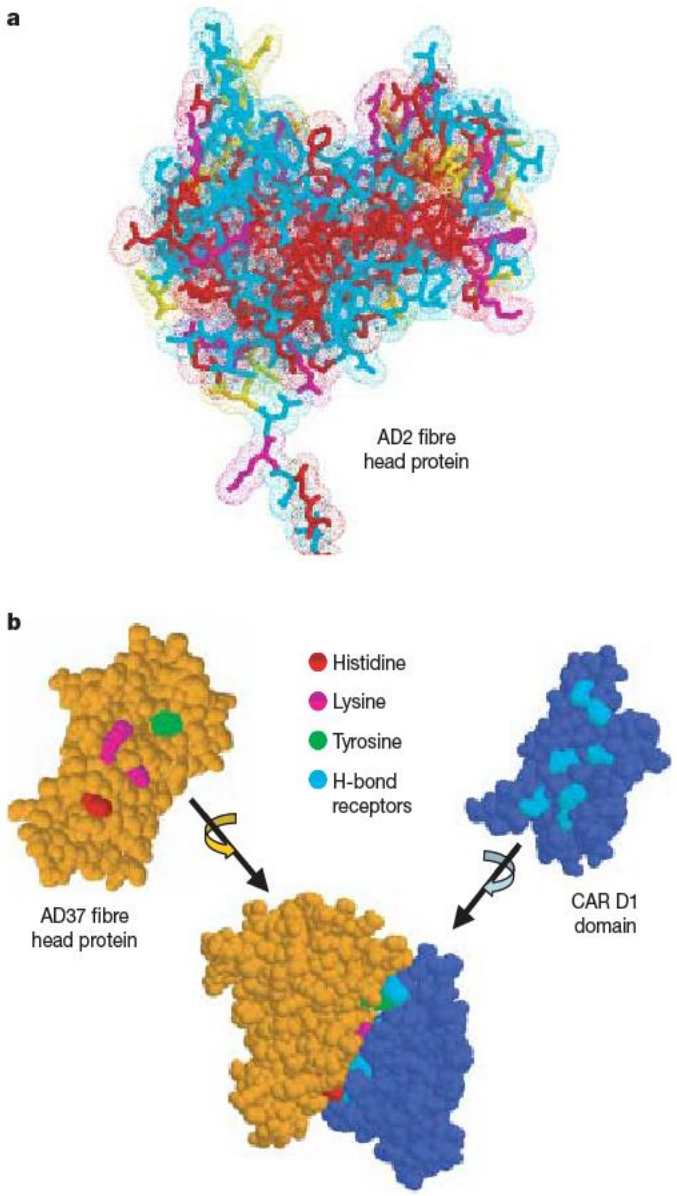

Figure 1 | Waterborne virus attachment head and receptor on host cell. **a**, Schematic of adenovirus-2 attachment fibre head showing amino acids with basic (magenta, orange, purple), acidic (yellow), and hydrophobic (red) side groups. **b**, Schematic of adenovirus-37 fibre head protein attaching to the D1 domain of the coxsackievirus-and-adenovirus receptor (CAR) on the host cell. Basic ionizable (histidine, lysine) and hydrophobic (tyrosine) side groups of AD37 amino acids and those in CAR D1 domain involved in hydrogen bonding are highlighted. The figure was developed with Protein Explorer[98] based on a structure described in ref. 99 (courtesy of Martin A. Page, University of Illinois).

Once these new materials are developed, they can be engineered into flow-through reactors for high-throughput systems. The configuration and associated cost of such systems could make them economically viable for applications ranging from large water-treatment plants supplying potable and non-potable water to point-of-use systems with segregated lines dedicated to human consumption and hygiene. Antiviral photocatalysts could be immobilized on fibres and foams of various materials[27–29], or incorporated into membranes[30]. Optical fibres could be used to bring photons into compact configurations such as monolithic reactors[31]. Reactors incorporating visible-light photocatalysts could be designed using sunlight as the source of photons[32,33], a configuration that would be particularly beneficial in developing countries. The resulting systems would provide a barrier against all pathogens by inactivating viruses and trapping any larger bacteria and protozoa cysts and oocysts with relatively high resistance to light and photocatalytic inactivation, all without producing DBPs or extensive use of chemicals.

Decontamination

The overarching goal for the future of decontamination is to detect and remove toxic substances from water affordably and robustly. Widely distributed substances, such as arsenic, heavy metals, halogenated aromatics, nitrosoamines, nitrates, phosphates, and so on are known to cause harm to humans and the environment. Two key problems are that the amount of suspected harmful agents is growing rapidly, and that many of these compounds are toxic in trace quantities. To detect their presence and remove them in the presence of safe and natural constituents that are 3 to 9 orders of magnitude more concentrated is challenging, expensive, and unreliable at present. Chemically treating the total volume of water to transform or remove a specific trace compound is also expensive and potentially itself harmful. Moreover, the treatment does not necessarily remove other harmful compounds, and safe constituents may interfere with the remediation. Thus, new methods to detect toxic compounds and decontaminate water are urgently needed.

The problems of detecting and accurately measuring toxic compounds in water and of selectively removing only these compounds are tightly linked. Both are affected by the particular combination of micropollutant classes (heavy metals, As(III/V), BTEX, pharmaceutical derivatives, agricultural chemicals, endocrine disrupters, and so on)[34] relevant to a specific water source. Furthermore, viable avenues for both detection and treatment are tied to the resource base available. Approaches to speciation of As(III/V) or elemental profiling relevant to western Europe are simply not an option for Bangladesh[35,36] or Benin[37]. Powerful methods of monitoring low concentrations of contaminants are invariably built around sophisticated laboratory instrumentation. It is extremely challenging to develop robust, low-cost, effective means of chemical sensing relevant to the water contamination problems of developing nations. Similarly, affordably treating toxic compounds in water, such as by

reducing As(III/V) concentrations to levels currently thought of as safe (<10 parts per billion), without producing toxic waste disposal issues has proved to be a major challenge. But although these goals are beset by severe technical difficulties, they also present exciting opportunities for the research community.

Speciation remains a challenging detection problem. For example, As(III) is estimated to be ~50 times more toxic than As(V), so both As(III) and total As must be measured. Anodic stripping voltammetry[38] has sufficiently low limits of detection (LOD = 1.2 $\mu g l^{-1}$) to be practical and is capable of measuring As(III) in the presence of large excesses of As(V). Alternatively, ion exchange separations may be combined with hydride generation atomic spectroscopy to measure As(III) and As(V) separately[39]. But neither method is suitable for untrained workers. These methods also demonstrate the related generic problem of the LOD dynamic reserve. The temptation, given that detailed dose–response data frequently do not exist (especially at low concentrations of toxic species), is to regulate to the existing analytical capabilities, which can create new problems. For example, if the total As concentration is regulated at a maximum contaminant level of 10 $\mu g l^{-1}$, then the 1.2 $\mu g l^{-1}$ LOD of As(V) represents only an eightfold dynamic reserve. It might not be possible to achieve a tenfold or greater dynamic reserve between the LOD and the maximum contaminant level using detection methods suitable for use by untrained workers to enhance human health.

Beyond these quantitative issues lies the dichotomy between the capabilities for detecting target compounds and for identifying potentially troublesome non-target species. Even powerful multidimensional analytical methods, such as liquid chromatography-mass spectrometry (LC-MS), struggle to characterize waters containing significant amounts of non-target species. These compounds must often be pre-concentrated by factors of 10^2 to 10^3 and can only be assayed accurately in the presence of a small number of potential compounds whose liquid chromatography retention behaviour is known[40]. Such problems point to the critical need to develop molecular recognition motifs (sensor reagents) that can be combined with micro-nanofluidic manipulation[41] and data telemetry to accomplish single-platform chemical sensing having the requisite figures of merit to be competitive with bench-scale instrumentation. In this regard the recent combination of catalytic DNA (DNAzyme) in a micro-nanofluidic platform is of considerable interest. Functional DNA, obtained through *in vitro* selection, can be used to bind metal ions with high affinity (yielding parts-per-trillion LODs) and specificity (>10^6-fold over other cations)[42]. When synthetically elaborated with proximal fluorophore and quencher, the resulting molecular beacon construct (see Fig. 2) may be placed in microfluidic formats to achieve the double selection of a chemical separation followed by a highly specific molecular recognition event[43]. Significant opportunities exist to exploit the *in vitro* selection process to achieve similar performance characteristics for a wide range of micropollutants.

Biosensing strategies are also beginning to be applied to waterborne pathogens. For example, capillary waveguide integrating biosensors have been applied to detect waterborne *Escherichia coli* O157:H7, an enterohaemorrhagic bacterium[44]. However, given the large fraction of the contaminated-water death toll that is due to waterborne pathogens, there is enormous potential for future development of bio-based measurement schemes.

Detection and remediation of toxic compounds are inextricably linked, as treatment of anionic micropollutants demonstrates. Determination of the anionic constituents of aqueous systems remains among the most challenging analytical problems. Typically, anions are determined by ion chromatography coupled with conductance detection, which is universal but does not have the sensitivity required in all instances. Sensitivity can be grafted on through the use of LC-MS[45] at considerable added expense, although lowering the limits of detection from the ~5 $\mu g l^{-1}$ level to ~0.05 $\mu g l^{-1}$ may well justify the cost. On the remediation side, compelling research opportunities surround is the development of high-specificity synthetic anion transporters although these have thus far been focused on biomedical applications[46]—for particularly refractory micropollutant species, such as ClO_4^- and NO_3^-. Anions also illustrate the complexity of designing effective treatment/remediation strategies. For example, disinfection of water sources with ozone (O_3) is highly effective, but if the water contains significant amounts of Br^-, oxidation to the problematic BrO_3^- takes place[47], effectively substituting one water contamination problem for another.

Similar strategic considerations affect the treatment versus removal decision. Whether to treat water via a chemical or biochemical conversion of a micropollutant to an innocuous form, or to remove the toxic contaminant via adsorption, chelation and filtration, or another method is a decision that rests largely on matching the problem to the sophistication of the available technology and the resource base to support the use of the technology, as well as how far the target concentration is below the maximum contaminant level. The use of Sono filter technology at local wells in Bangladesh and reverse osmosis (RO) systems in central plants in the USA may both represent optimized solutions for As removal within the context of the local problem[48]. However, here, too, opportunities exist for research to make an impact.

The treatment protocols used widely and envisioned for the future all encompass a complex interplay of elementary steps such as transport, partitioning, reaction and conversion, and release. To this end, fundamental advances in understanding these processes will necessarily involve sophisticated modelling to assess the way in which the basic steps are coupled most effectively[49], and modelling-based predictions of potential removal activity[50]. Modelling is essential to optimize multi-step strategies—for example, the capture of As by monodisperse Fe_3O_4 nanocrystals followed by magnetic separation of the waste stream[51]—which are often the most effective, or perhaps the only, possible approaches.

Another critical problem involves unintended transformations of non-targeted pollutants. For example, treatment of wastewater with

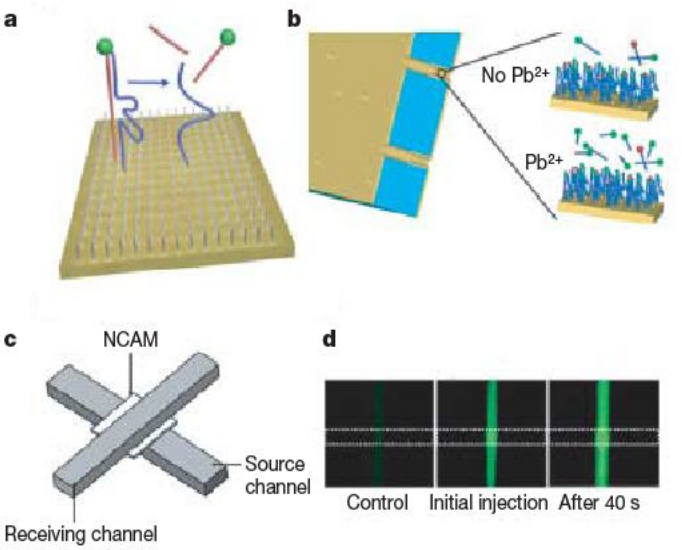

Figure 2 | Lead DNA sensor with a micro-nanofluidic device. Immobilized DNAzyme sensor and micro-nanofluidic devices for detection of Pb^{2+} by fluorescently labelled 17E DNAzyme. **a**, Schematic of immobilized DNAzyme showing catalytic beacon signalling of reaction on the surface, releasing fluorophore into solution for detection. **b**, Schematic of DNAzyme immobilized within the pores of a nanocapillary array membrane (NCAM) with inset showing the mode of ratiometric fluorescence signalling in the absence or presence of Pb(II). **c**, Schematic representation of orthogonal microfluidic channels separated by a NCAM flow gate. **d**, Fluorescence micrographs of the receiving channel before injection of Pb^{2+} sample into a receiving channel containing ~1 μM 17E DNAzyme, after initial Pb^{2+} injection and after 40 s of total injection time for a DNAzyme–NCAM microfluidic device.

Cl_2 or monochloramine can oxidize dimethylhydrazine to the suspected carcinogen *N*-nitrosodimethylamine (NDMA). These unintended secondary effects would seem to argue for separation over transformation strategies, but the relative cost and effectiveness of each approach needs to be considered on a case-by-case basis. A promising workaround focuses on exploiting biology to effect either transformation, such as the biodegradation of NDMA by monooxygenase-expressing microorganisms[52], or removal, as exemplified by the Fe-specific siderophile desferrioxamine-B produced by *Streptomyces pilosus*. Desferrioxamine-B exhibits stability constants in excess of 10^{26} for Th(IV) and Pu(IV), and so may be useful in actinide remediation strategies[53]. Of course, organism-oriented strategies must also be vetted to ensure that they do not introduce other undesirable secondary effects.

Finally, an ubiquitous problem in remediation strategies is the cost or use of critical components that are consumed in stoichiometric reaction, which spurs interest in catalytic treatment approaches to convert organic compounds to innocuous N_2, CO_2 and H_2O. Major anion pollutants such as nitrates and perchlorates are now removed via ion exchange resins or RO, leaving a deleterious brine to be disposed of. Next-generation remediation may use bi-metallic active catalysts to mineralize the brine, such as Pd-Cu/γ-alumina catalysed reduction of NO_3^- (ref. 54). Future efforts may include incorporating active nanocatalysts in a membrane barrier to transform anions at low concentrations in a hybrid process. The combination of modelling and experiments can reveal the mechanisms of these reduction reactions, helping to identify potentially transformative catalytic remediation strategies.

Re-use and reclamation

The overarching goal for the future of reclamation and re-use of water is to capture water directly from non-traditional sources such as industrial or municipal wastewaters and restore it to potable quality. Of all the water withdrawn from rivers, lakes and aquifers, the majority is returned to the environment. Agricultural and livestock users return the least at ~30–40%, whereas industrial users return ~80–90%, power generation returns considerably more at ~95–98%, and public and municipal users return ~75–85%. The rest is lost to the atmosphere or is consumed in biological or chemical processes. A large part of the cost of water for human use is pumping, transport and storage (particularly in developing countries whose citizens often spend substantial time acquiring water). Thus recovering water at or close to the point of use should be very efficient. However, unlike the decontamination of trace compounds just discussed, wastewater contains a wide variety of contaminants and pathogens, and has a very high loading of organic matter, all of which must be removed or transformed to harmless compounds.

Municipal wastewaters are commonly treated by activated sludge systems that use suspended microbes to remove organics and nutrients, and large sedimentation tanks to separate the solid and liquid fractions. This level of treatment produces wastewater effluent suitable for discharge to surface waters or for restricted irrigation and some industrial applications. Similarly, biological treatment via traditional trickling filters and aquacultures have been used extensively to reduce solids and remove ammonia and nitrites from water. Typically, these biological treatment systems are large with long water residence times. A technology now actively being pursued is membrane bioreactors (MBRs)[55–57]. This technology combines suspended biomass, similar to the conventional activated sludge process, with immersed microfiltration or ultrafiltration membranes that replace gravity sedimentation and clarify the wastewater effluent. MBRs can produce high-quality effluent that is suitable for unrestricted irrigation and other industrial applications.

MBRs have also the potential for use in developing countries to address the pressing need for improved sanitation[55]. Possible applications in developing countries include the direct treatment of raw sewage, particularly in rapidly growing megacities, and the extraction of valuable resources from sewage, namely clean water, nutrients (mostly N and P), and energy. The small footprint, flexible design, and automated operation of MBRs make them ideal for localized, decentralized sewage treatment in the developing world.

One of the growing applications of MBRs is as pretreatment for RO, which, when followed by UV disinfection (or, potentially, visible-light-activated photocatalysts), can produce water for direct or indirect potable use (Fig. 3). Current wastewater re-use systems use a conventional activated sludge process, followed by a microfiltration MBR pretreatment of the secondary effluent, which has high quantities of suspended and dissolved solids. The effluent water from the MBR still partially contains dissolved species and colloidial substances that act to foul the membranes of the subsequent RO system used as a final barrier to contaminants in the product water. Employing a 'tight' ultrafiltration membrane in the MBRs lets through fewer dissolved solids than does microfiltration, allowing the RO system to operate with significantly less fouling. Futuristic direct re-use systems envisioned involve only two steps: a single-stage MBR with an immersed nanofiltration membrane (obviating the need for an RO stage), followed by a photocatalytic reactor to provide an absolute barrier to pathogens and to destroy low-molecular-weight organic contaminants that may pass the nanofiltration barrier.

A major obstacle to the efficient application of MBRs in current or next-generation re-use systems is membrane fouling, particularly when it leads to flux losses that cleaning cannot restore[56,58]. Fouling in MBRs is primarily caused by microbe-generated extracellular polymeric substances, most notably polysaccharides, proteins and natural organic matter. The development of economical, high-flux, non-fouling membranes is therefore needed before viable MBR processes, as well as other membrane-based approaches for wastewater reclamation, can be achieved.

Fouling of polymer membranes is influenced by membrane chemistry and morphology. Polymers used in porous membrane manufacture have chemical and mechanical stability, but are generally hydrophobic in nature, and as a consequence are highly susceptible to adsorption of organic foulants. Commercial methods to reduce fouling largely involve graft polymerization of hydrophilic monomers on the membrane surface[59]. The resulting 'brush' of hydrated

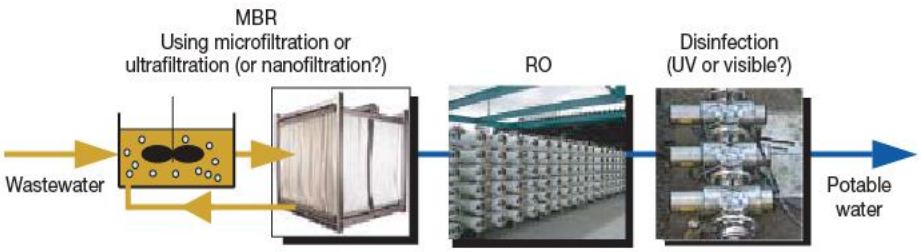

Figure 3 | Membrane bioreactor treatment system for direct conversion to potable water. Depiction of a next generation MBR-based treatment method that can potentially take wastewater from municipal, agricultural, livestock or other high-organic-content sources and convert it to potable water. Future methods may be able to omit the RO step with a nanofiltration membrane, and follow with a visible light disinfection step to ensure that all pathogens, including viruses, are inactivated.

chains serves as a steric-osmotic barrier to foulant adsorption, but reduces intrinsic permeability owing to partial blocking of surface pores, while internal pores may go unmodified and remain prone to fouling[60]. The extra manufacturing steps also add to membrane cost.

Alternative *in situ* approaches to membrane surface modification under development may generate more efficacious brush layers without the drawbacks of surface graft polymerization[61–64]. Comb copolymers, having hydrophobic backbones and hydrophilic side chains, function as macromolecular surfactants when added to membrane casting solutions[62], lining membrane surfaces and internal pores during the conventional immersion precipitation process used in membrane manufacture. Order-of-magnitude flux enhancements[63,65] and complete resistance to irreversible fouling by the three classes of extracellular polymeric substance foulants[65,66], recently demonstrated for such ultrafiltration membranes (see Fig. 4), offer substantial promise for decreasing operational costs of wastewater treatment through reduced membrane cleaning and replacement and increased process efficiency.

Next-generation membranes offer further opportunities for improved contaminant retention or recovery of valuable constituents from wastewaters, without intensive chemical treatment and while reducing the need for subsequent decontamination. These advanced filtration processes require membranes with much narrower pore size distributions than those derived from immersion precipitation, in addition to fouling-resistant surface/pore chemistries[61,67]. Approaches under investigation include block copolymers, graft/comb copolymers, or lyotropic liquid crystals that self-assemble to form nanodomains that are highly permeable to water[68–71], or can be selectively removed to create nanopores for water passage[72,73]. Such nanostructured materials may be implemented as thin-film coatings on conventional ultrafiltration or microfiltration membrane supports[70,72,74], or on novel high-flux base membrane structures, such as electrospun nanofibres[75]. Recently, for example, rigid star amphiphiles with 1–2 nm hydrophobic cores and hydrophilic side chains were coated onto polyethersulphone ultrafiltration membranes to obtain nanofiltration membranes with comparable or better rejection of As(III) and water permeability several times greater than commercial nanofiltration membranes[76]. The commercial viability of this new class of thin-film composite membranes for water re-use hinges on the development of inexpensive coatings, chemistries and scalable processing methods that can reproducibly achieve the desired membrane structure and yield fluxes comparable to today's ultrafiltration membranes.

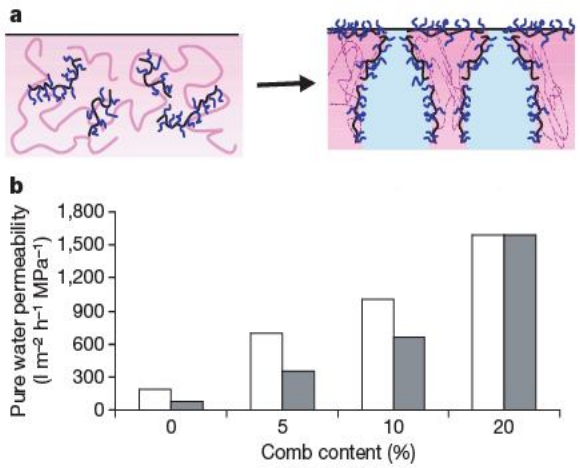

Figure 4 | Comb copolymer amphiphiles for fouling-resistant membranes. **a**, Schematic illustration of *in situ* approach using comb copolymer amphiphiles to modify ultrafiltration membrane surfaces and internal pores during membrane casting. **b**, Pure water permeability of polyacrylonitrile ultrafiltration membranes incorporating 0–20% comb copolymer additive having a polyacrylonitrile backbone and polyethylene oxide side chains. White bars show the initial pure water permeability, and grey bars show the pure water permeability after 24 h of dead-end filtration of 1,000 mg per litre of bovine serum albumin in phosphate buffered saline, followed by a deionized water rinse. Initial flux and flux recovery increase with comb additive content. Membranes exhibit complete resistance to irreversible fouling at 20% comb content (data from ref. 65).

Desalination

The overarching goal for the future of desalination is to increase the fresh water supply via desalination of seawater and saline aquifers. These sources account for 97.5% of all water on the Earth, so capturing even a tiny fraction could have a huge impact on water scarcity. Through continual improvements, particularly in the last decade, desalination technologies can be used reliably to desalinate sea water as well as brackish waters from saline aquifers and rivers.

Desalination of all types, though, is often considered a capital-[77] and energy-intensive[78] process, and typically requires the conveyance of the water to the desalination plant, pretreatment of the intake water, disposal of the concentrate (brine), and process maintenance. Estimated costs of pumping from sea intake to the desalination plant vary widely with geographical location, height and distance from the source water[77]. But so far, the total cost and increased environmental concerns have limited the widespread adoption of desalination technologies. Nevertheless, for a state-of-the-art RO system that uses as little as ~2.2 kW h of electrical energy to produce a thousand litres of drinking water inside the desalination plant, the total energy usage to desalinate water will be ~0.005 kW h l^{-1}, which includes the electrical plus modest conveyance energy needs. Putting this estimated energy use into perspective reveals that supplying even 50 litres a day per capita of drinking water at 0.25 kW h can be a small fraction of the daily energy required per capita (ranging from 3.2 kW h in China to 30 kW h in the USA) for living in a world with strained environmental resources (see http://telstar.ote.cmu.edu/environ/m3/s3/02needs.shtml).

The major desalination technologies currently in use are based on membrane separation via RO and thermal distillation (multistage flash and effect distillation), with RO accounting for over 50% of the installed capacity[77,78]. Conventional thermal desalination processes are inefficient in their use of energy and suffer particularly from corrosion, as well as scaling that also affects RO. Even where fuel is readily available and low-cost, high capital and operational costs limit adoption. Therefore, the market share of large conventional thermal desalination plants will probably decline. However, for family and very small community systems in remote locations, especially in the developing world, solar thermal distillation and humid air desalination technologies may find an increasing role, particularly in inland semi-arid areas with access to saline lakes and aquifers[79,80]. These thermal technologies may also find small-scale applications in locations without ready sources of energy, other than solar.

Although RO systems have a relatively low rate of energy consumption, they use high-cost electrical energy. RO desalination, however, can take advantage of low-grade heat energy to increase flux through the membrane for a given pressure drop. This thermally enhanced RO finds applications in locations where such heat energy is available, typically using waste heat from a co-located electrical power generation and RO desalination plant. We also envision hybrid desalination plants that would combine thermally enhanced RO and thermal desalination to lower electrical energy consumption per unit of product water further, while achieving higher water recoveries than can RO alone. Desalinating inland saline waters, which are present on most continents in quantities similar to fresh water, can also be used to increase water supplies, but disposal of the residual concentrate is a major problem. Hybrid desalination technologies that concentrate precipitates and salts while extracting the water with membranes can potentially process the brine. Two alternative desalination technologies currently under investigation, forward osmosis[81] and membrane distillation[82], can also use low-grade heat

energy and may be used alone, or as hybrid systems with RO, to achieve high water recoveries.

For large-scale desalination, RO has advanced significantly in the past decade, particularly owing to the development of more robust membranes and very efficient energy recovery systems. As a result, the reduction in energy consumption of RO desalination has been remarkable[77,78,83,84]. The specific (per unit of produced potable water) energy of desalination has been reduced from over 10 kW h m^{-3} in the 1980s to below 4 kW h m^{-3} (refs 78, 83). Any desalination system will be most energy efficient if it involves a reversible thermodynamic process, which is independent of the system and mechanisms used.

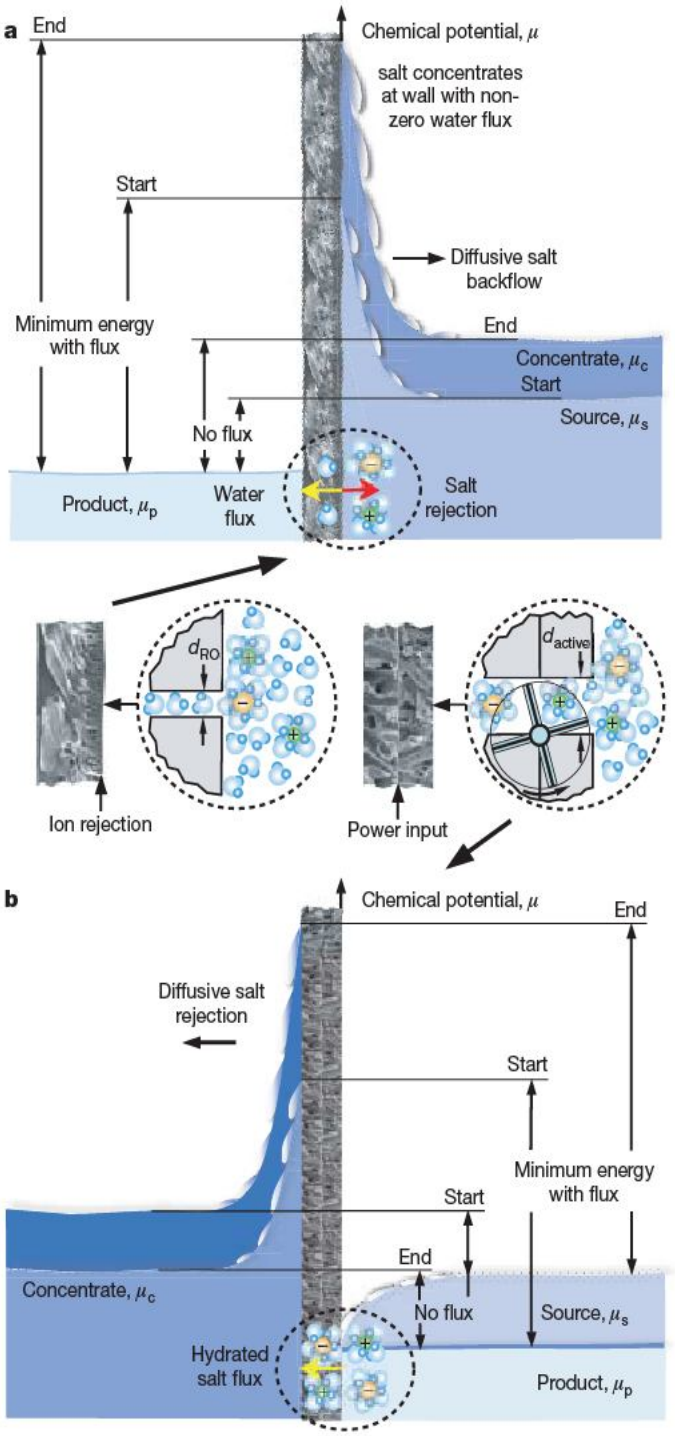

Figure 5 | Reverse osmosis and active desalination membrane processes. Concentration gradients in RO (**a**) and active (**b**) desalination membranes. The energy levels marked with 'start' and 'end' correspond to the evolution of each process. The darker blue colour denotes higher concentration. Insets depict different mechanisms of salt ion separation. The active process with energy input shows a conceptual strategy for overcoming the Born barrier with fixed charges.

From the free-energy change on removing a small amount of pure water from a mixture of water and salt, the theoretical lower bound of the energy needed for desalination can be estimated[85]. For zero per cent recovery, that is, the removal of a relatively small amount of water from a very large amount of sea water, the calculated theoretical minimum energy for desalination is 0.70 kW h m^{-3} of fresh water produced. This theoretical minimum increases to 0.81, 0.97 and 1.29 kW h m^{-3} for recoveries of 25, 50 and 75%, respectively, suggesting that further improvements in the energy efficiency of RO desalination are still possible.

Although RO is currently the state-of-the-art desalination technology, there are several challenges and opportunities that could result in additional reductions in the total cost per unit of product water. Among the major challenges of RO desalination are membrane fouling, relatively low recovery for sea water desalination (less than ~55%), which results in large volumes of concentrated brine, and relatively low removal of low-molecular-weight contaminants, most notably boron in sea water. Future RO desalination membranes will ideally have high water flux per unit of pressure applied, near-complete rejection of dissolved species, low fouling propensity, and tolerance to oxidants used in pretreatment for biofouling control. The total cost for RO involves three strongly interrelated components that depend upon region, source water, and energy sources: capital (infrastructure, equipment, membrane replacement), energy (thermal and electrical), and operation (pretreatment, post-treatment, concentrate disposal and cleaning). Lowering the flux to save energy may be offset by the consequent increase in capital costs. Increasing the permeability of the RO membranes decreases both capital and energy costs, but may increase the cost for pretreatment and cleaning. Improvements must be made in all three components to lower the total cost of the product water.

Recent work by the Affordable Desalination Coalition[78,84] has demonstrated a remarkably low specific energy of seawater desalination, at 1.58 kW h m^{-3}, under ideal conditions (that is, new membranes, no fouling, and low water flux) at 42% recovery. This value is relatively close to the theoretical minimum energy for seawater desalination at that recovery, suggesting that next-generation fouling-resistant RO membranes will be able to desalinate sea water with lower energy consumption. Asymmetric membranes depicted in Fig. 5 (a) currently used for RO have relatively large random pore size distributions. Therefore, the separation layer is thicker than ideal to ensure adequate salt rejection, reducing the flux rate. Desalination typically involves ions with small hydration diameter d_H, which require pores with a hydraulic diameter of $d_{RO} < d_H$ to exclude them, increasing mainly enthalpic energy requirements. The energy of desalination depends critically on pore diameters, and the chemical affinity of water and ions with the pore wall.

Approaching the theoretical minimum energy is impractical for desalination plants, because it would require huge facilities with high capital costs. Moreover, in real desalination processes, energy is lost because of inherent thermodynamic irreversibilities that arise from diffusion, viscous dissipation and flux-rate-dependent losses. To reduce the energy needed for desalination, the rate of entropy generation Φ must be minimized because the energy consumed by irreversible processes is $\sim T\Phi$, where T is the absolute temperature. Φ can be expressed as $J_v\Delta p + J_d\Delta\pi + J_a\Delta a$, where J_v is the total volumetric flux of water plus solute, J_d is the flux of solute relative to the water, Δp is the pressure difference from frictional losses ($\Delta p \propto J_v$), $\Delta\pi$ is the osmotic pressure difference across the membrane, and $J_a\Delta a$ is the entropy generation from any active ion pumping. The higher the flux, the higher the salt concentration will be at an RO barrier, increasing $\Delta\pi$ and Δp. Interestingly, for the same separation performance (that is, the same $J_d\Delta\pi$), if $J_a\Delta a$ is sufficiently small, the entropy generation using active membranes could be less than for RO for a water recovery of much more than 50%, because the fluid flux in RO will be higher than in active transport ($J_{v|RO} \gg J_{v|active}$), and the

diffusion of salts driven by concentration gradients will act in favour of the separation.

Membranes with a uniform pore distribution and a more permeable separation layer can potentially maintain or improve salt rejection while increasing the flux in RO. Recent research on the transport of water through hydrophobic double-walled carbon nanotubes is promising, demonstrating water fluxes that are over three orders of magnitude higher than those predicted from continuum hydrodynamic models (refs 86–89; also O. Bakajin, personal communication, 23 October 2007). The high flux may be due to the carbon nanotubes' atomically smooth, hydrophobic walls allowing considerable slip of water through the pores. The preliminary work of ref. 89 reported unusually high water flux through microfabricated membranes comprised of aligned carbon nanotubes ~3 μm long with an inner diameter of ~1.6 nm. Further measurements with these membranes reveal[90] salt rejection coefficients that match or exceed those of commercially available nanofiltration membranes, while exceeding their flux by up to four times. But such membranes may be difficult and costly to manufacture, prone to defect formation, and might have a high propensity for fouling given their hydrophobic nature.

The high performance of membranes based on carbon nanotubes[86,87,89], however, reveals an important pore characteristic shared by biological ion channels: hydrophobic pores ~1 nm in diameter. These cores allow the ion hydration shell to remain intact, thereby reducing the enthalpic translocation energy to be closer to the entropic loss for confining an ion in a pore. Decreasing the pore diameter much below 1 nm creates a large free-energy barrier, which arises from stripping the hydration shell off the ion and water molecules that need to overcome a Born energy barrier. Modifying the surfaces of the membrane, as discussed for nanofiltration membranes, can alter the surface properties, and thus potentially decrease the energy barrier.

Technological challenges to incorporating carbon nanotube materials include the functionalization of the mouth of the pores to increase selectivity and potentially reduce hydrophobicity at the surface, integration of the active layer with robust support substrates, scaling up the fabrication of the ion channel and carbon-nanotube-based membranes and increasing the pore density per area of the active layer, and decreasing the cost of membrane fabrication. Still, the costs of such membranes could eventually be affordable with future improvements in carbon nanotube synthesis and membrane processing.

Aquaporins (water channels) and ion channels of biological cells have also motivated the search for alternative approaches to engineering membranes with high water flux and selectivity[91,92]. *De novo* synthesis of ion channels[93] and the development of low-molecular-weight anion transporters is an emerging topic in supramolecular chemistry[94]. Based on an array of aligned carbon nanotubes with hollow graphitic cores embedded within a solid polymer film, the first biomimetic protein channel controlled by the same mechanism of phosphorylation/dephosphorylation that occurs in nature has also been recently reported[95]. However, much work remains to incorporate these futuristic materials into large-area membranes at competitive costs.

Even if a perfect membrane could be created, with no pressure drop required for complete salt rejection, the increase of flux rates for RO is ultimately limited by the concentration polarization layer at the membrane (see Fig. 5a), which constitutes an additional impedance to fluid flow. The higher the flux of water, the higher the gradient in solute concentration on the rejection side. The polarization impedance can be reduced via tangential fluid flow, but can never be eliminated. What is worse, the transmembrane chemical potential difference increases along the direction of the tangential flow (from 'start' to 'end' in Fig. 5a), while the transmembrane pressure difference decreases because of pressure losses, resulting in additional irreversible losses with higher fluxes.

To see how active systems might compare to RO systems, we can extrapolate from the energetics of existing ion channels. Biological channels transfer 10^7 ions per pore per second, and measurements corroborated by systematic computer simulations reveal that the free-energy barrier for biological potassium channels is 2–5 kcal mol^{-1} (depending on the type of K^+ channel), which corresponds to a specific energetic requirement of 2.55–6.4 kW h m^{-3} of water produced from sea water with a salt concentration of 32,000 parts per million. For potassium ions, the lower bound is near the current energetic costs for RO, but is still much higher than the theoretical minimum. However, these channels are ion- and charge-specific, and there is a significant energetic cost for the exclusion of the other ions. If active nanopores of dimensions greater than 1 nm are created that pass a multiplicity of anions and cations, as depicted in Fig. 5b, the energetics can potentially drop by more than a factor of two below that of biological channels.

Bio-inspired systems for active transport provide another route towards improving the energetics of desalination. In contrast to conventional desalination whereby water is 'pushed' through an RO membrane by a pressure gradient, or in electrodialysis whereby hydrated anions and cations are forced through their respective ion-selective membranes by electrokinetic action, active ion separation involves pumping of both hydrated anions and cations through the same membrane via modulation of pore potentials, against a chemical potential, leaving desalinated product water. As Fig. 5b illustrates, membranes that actively 'pull' hydrated ions through the barrier reverse the direction of the concentration polarization layer, and should not suffer the same decrease of performance with increasing flux as does RO. Nature also provides a solution to the problem of lowering the cost of overcoming electrostatic barriers in engineered systems, which typically involve dielectric membranes. The Born energy barrier[96] to move an ion of charge q from water to the low-dielectric-constant membrane ($\varepsilon \approx 2$ for typical biological membranes) is $\Delta G \approx q^2/d_H(1/\varepsilon - 1/80)$. This barrier can be offset by the energy liberated if the penetrating ion meets a counter-ion buried inside the membrane ($\Delta G' = -q^2/\varepsilon d_H$), or by the pump, as depicted in the insets of Fig. 5. It has been suggested[97] that for biological ion pumps the energy cost of burying the counter ion is paid for by actively manipulating charged groups in the proteins within the pumps. However, whether passive or active, high-permeability membranes with high resistance to fouling are needed, as well as new strategies for synthesizing membranes with multiple functions to screen small molecules, and to resist stresses and chemical degradation.

Conclusion

The work highlighted here, plus the tremendous amount of additional research being conducted on every continent that could not be mentioned, is sowing the seeds of a revolution in water purification and treatment. We believe that advancing the science of water purification can aid in the development of new technologies that are appropriate for different regions of the world. That said, the sheer enormity of the problems facing the world from the lack of adequate clean water and sanitation means that much more work is needed to address the challenges particular to developing nations, which suffer a diversity of socio-economical-political-traditional constraints, and require a broader approach incorporating sustainable energy sources and implementing educational and capacity building strategies. Consortiums of governments at all levels, businesses and industries, financial and health organizations, water and environment associations, and educational and research institutions need to focus increasing attention towards solving these water problems. While better water resource management, improved efficiencies, and conservation are vital for moderating demand and improving availability, it is our belief that improving the science and technology of water purification can help provide cost-effective and robust solutions.

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Acknowledgements We acknowledge the US National Science Foundation Science and Technology Center, *WaterCAMPWS*, Center for Advanced Materials for the Purification of Water with Systems.

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ARTICLES

Ancient, highly heterogeneous mantle beneath Gakkel ridge, Arctic Ocean

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The Earth's mantle beneath ocean ridges is widely thought to be depleted by previous melt extraction, but well homogenized by convective stirring. This inference of homogeneity has been complicated by the occurrence of portions enriched in incompatible elements. Here we show that some refractory abyssal peridotites from the ultraslow-spreading Gakkel ridge (Arctic Ocean) have very depleted $^{187}Os/^{188}Os$ ratios with model ages up to 2 billion years, implying the long-term preservation of refractory domains in the asthenospheric mantle rather than their erasure by mantle convection. The refractory domains would not be sampled by mid-ocean-ridge basalts because they contribute little to the genesis of magmas. We thus suggest that the upwelling mantle beneath mid-ocean ridges is highly heterogeneous, which makes it difficult to constrain its composition by mid-ocean-ridge basalts alone. Furthermore, the existence of ancient domains in oceanic mantle suggests that using osmium model ages to constrain the evolution of continental lithosphere should be approached with caution.

The Earth's mantle is known to be chemically heterogeneous on spatial scales ranging from the size of ocean basins down to kilometres or possibly metres, with incompatible-element-enriched portions surviving up to 3 Gyr (billion years)[1–5]. However, on the basis of the relatively uniform radiogenic isotope and trace element composition of normal mid-ocean-ridge basalts (N-MORB), the depleted MORB mantle (DMM) source has often been invoked as a large-scale homogeneous and degassed reservoir[3,6–8]. This homogeneity has been complicated by the mixing of the primary depleted basalts (N-MORB) with enriched basalts (E-MORB)[1–4], and also by the recognition of ancient depletion inherited in some MORB[9,10] and abyssal peridotites[11,12]. Geochemical studies of MORB largely revolve around elements that preferentially enter the melt phase during partial melting (that is, incompatible elements). Once clinopyroxene is exhausted during partial melting, these refractory peridotites cannot be re-melted under normal anhydrous conditions and thus a portion of the mantle is rendered invisible in magmas derived from any subsequent melting event. The refractory mantle domains are more likely to be preserved and detected at mid-ocean ridges with low spreading rates, because they are not obscured by melting processes as at other faster spreading ridges. Gakkel ridge is an ultraslow-spreading ridge and magmatic activity is relatively weak there[13,14]. Fresh abyssal peridotites recovered from Gakkel ridge[15] thus provide us an opportunity to study ancient depletion signatures inherited in the refractory mantle.

Ancient depletion signals in refractory mantle domains are well recorded by the Re–Os isotopic system, because of its unique geochemical properties. Unlike other long-lived isotopic systems (for example, Sm–Nd and Rb–Sr) in which both parent and daughter elements are lithophile, osmium is chalcophile and behaves as a compatible element in mantle peridotites during mid-ocean-ridge partial melting. Rhenium, on the other hand, behaves as a moderately incompatible element with a bulk partition coefficient similar to that of aluminium; even different phases probably control the partitioning of these two elements[16]. Furthermore, higher Os concentrations in peridotites relative to basaltic melts render the Os isotopes in peridotites more robust to late-stage metasomatism and contamination processes than lithophile isotope systems. Previous Re–Os studies on abyssal peridotites have indicated that some mantle domains retain evidence for ancient partial melting events[11,12,17].

Geological setting of samples

The 1,800-km-long Gakkel ridge is the slowest-spreading mid-ocean ridge in the world. It forms the North America/Eurasia plate boundary in the Arctic Ocean (Fig. 1). To the west it passes via the Lena trough and the Molloy fracture zone into the Knipovich ridge, and to the east it runs into the Siberian continent as a broad region of continental rifting on the Laptev shelf[14]. The spreading of Gakkel ridge propagates eastwards with a rate varying from 14.6 mm yr^{-1} on its western end to 6.3 mm yr^{-1} at its Siberian eastern end[14,18]. The ridge axis is continuous, with no transform offsets, and there are three main tectonic zones, from west to east, the Western Volcanic Zone (7° W to 3° E), the Sparsely Magmatic Zone (3° E to 29° E) and the Eastern Volcanic Zone (29° E to 85° E)[14]. Samples in the present study are selected from two dredge hauls, HLY0102-D70 (AMORE 2001)[14] and PS66-238 (ARK XX-2, 2004)[15] (Fig. 1). Dredge haul PS66-238 is located at the transition from the Western Volcanic to the Sparsely Magmatic Zone (84° 29.23′ N, 4° 12.64′ E), around which no basalts, only peridotites, have been recovered (3° E to 8° E), and it is thought to indicate an amagmatic segment. Dredge haul HLY0102-D70 represents the easternmost occurrence of peridotite found on Gakkel ridge so far and is located near a volcanic centre in the Eastern Volcanic Zone. The two dredge hauls straddle an important geochemical boundary, indicated by the radiogenic compositions of MORB samples globally between western Gakkel DUPAL-like mantle and eastern Gakkel mantle, which is similar in nature to that of the north Atlantic[19].

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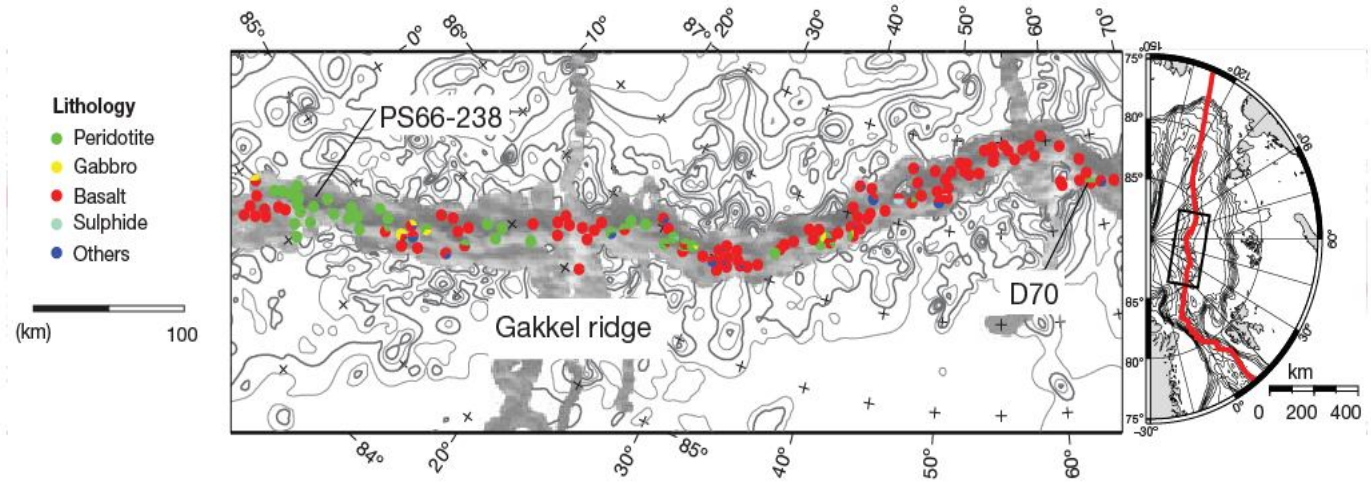

Figure 1 | Sketch map of dredge hauls on Gakkel ridge. Dredge haul PS66-238 is located at the transition from the Western Volcanic Zone to the Sparsely Magmatic Zone, in which only peridotites and no basalt have been recovered. Dredge haul D70 is recovered in the Eastern Volcanic Zone, which is the easternmost occurrence of peridotite found on Gakkel ridge as yet.

The samples from dredge PS66-238 comprise nine spinel lherzolites, one plagioclase lherzolite and one harzburgite. All of the samples from dredge PS66-238 are extremely fresh but with centimetre-thick brown-yellow weathering rims, which were separated from their fresh interiors. Most interior portions have a loss on ignition (LOI, an indicator of volatile components in the rock due to olivine serpentinization) close to 0% and only two of them are around 1%. Their weathered rims have slightly higher LOI (up to 3%) relative to their corresponding interiors, but these are still substantially lower than those in typical serpentinites (~14%). Samples from dredge D70, consisting of six lherzolites and one harzburgite, are typical serpentinized peridotites with large LOI (9–14%). Most of the peridotite samples from both dredges are relatively fertile with similar ranges of bulk Al_2O_3, 1.3–3.2% in D70 and 1.5–3.1% in PS66-238 (see Supplementary Table 1). Both D70 and PS66-238 samples have a similar range of chromium number, Cr# = molar Cr/(Cr+Al), in their residual spinels (~0.12–0.28 and ~0.15–0.28, respectively), which indicates that they were subjected to low degrees of partial melting[20] (Fig. 2a). The same conclusion can also be derived from the content of ytterbium (Yb) in the clinopyroxene (cpx). Clinopyroxenes have higher light rare earth elements, indicated by high Ce/Yb ratios, than predicted by simple partial melting models (Fig. 2b), implying late-stage enrichments after low-degree partial melting[21].

Re–Os isotopes of Gakkel abyssal peridotites

Except for one sample, all PS66-238 samples have higher Os concentrations than the D70 samples (Fig. 3a). This cannot be accounted for by any serpentinization effect because the more serpentinized sample rims are not systematically higher than the less serpentinized interiors; thus it may either be a source characteristic or reflect the different magmatic activity in the mantle beneath these two dredge hauls. Rhenium in the peridotites is potentially affected by secondary processes: that is, serpentinization and melt refertilization. No large gap in Re content, however, exists between the two dredge hauls, all of which are generally lower than the estimated value of the primitive mantle (0.34 parts per 10^9, ref. 22).

Replicate analyses indicate that Os contents are variable (Supplementary Table 1), because of a 'nugget effect' from different amounts of small sulphide phases in the sample aliquots, but that Os isotopes are highly reproducible. Previous studies have suggested that sulphides in mantle peridotites can be very heterogeneous in Os isotopic composition on the individual grain scale, from relatively depleted in the silicate-included sulphides to highly radiogenic in the interstitial sulphides[23]. The highly reproducible Os isotopes but variable Os concentrations in replicate analyses indicate that sulphides in Gakkel abyssal peridotites (both serpentinized and fresh) have similar Os isotopic compositions. Notably, $^{187}Os/^{188}Os$ ratios of samples from both dredge hauls have similar ranges, varying from depleted to relatively radiogenic values, despite their substantial differences in Os contents (Fig. 3a).

Neither the D70 nor the PS66-238 samples show any correlation between their Re/Os and Os isotopic ratios, whereas a good correlation exists between Al_2O_3 and $^{187}Os/^{188}Os$ in the D70 samples (Fig. 3b). Rhenium is more likely to be disturbed by late processes than aluminium, and this is the reason that bulk Al_2O_3 content is often used as a proxy for Re/Os to calculate the ages of partial melting events in mantle peridotites[24]. Although the PS66-238 samples have similarities with D70 samples in both bulk Al_2O_3 contents and $^{187}Os/^{188}Os$ ratios, an Os–Al_2O_3 correlation does not exist in PS66-238 samples (Fig. 3b). However, the most refractory sample does have the lowest $^{187}Os/^{188}Os$.

Ancient and recent partial melting

The correlation between $^{187}Os/^{188}Os$ and Al_2O_3 was first observed in orogenic peridotites[24], and has subsequently also been reported in the continental mantle from other tectonic settings, for example mantle xenoliths[25–27]. Until now, no such relationship has been found in the young asthenospheric mantle, that is, abyssal peridotites. This could be attributed to the convective stirring in the oceanic mantle, the juxtaposition of pieces of mantle with different melting histories, and subsequent partial melting and reactive melt migration beneath the ridge. The inherited depletions in Gakkel ridge abyssal peridotites record ancient melting events, which extracted Re and caused their unradiogenic $^{187}Os/^{188}Os$ ratios. Inherited ancient partial melting has also been previously invoked as the cause of unradiogenic $^{187}Os/^{188}Os$ ratios in abyssal peridotites from the Mid-Atlantic Ridge[11,12] and in forearc mantle peridotites from the Izu–Bonin–Mariana subduction zone[17]. Ancient depletions have also been inferred to exist in the mantle sources of some MORB[9,10].

Another possibility is that the D70 samples with a correlation between $^{187}Os/^{188}Os$ and Al_2O_3 represent a residual subcontinental lithospheric mantle, which is delaminated and incorporated into asthenosphere[28,29]. This is unlikely, however, because studies on MORB from the eastern Gakkel ridge suggest that they come from normal depleted 'Atlantic-like' oceanic mantle, and no signature of any continental mantle in their mantle source has been found in these MORB[19].

Harzburgites from both dredge hauls (D70-62 and PS66-238-22) give the oldest Re depletion ages yet found in global abyssal peridotites, around 2.2 Gyr (relative to primitive upper mantle[30]). These ancient depletion events are far older than, and therefore unrelated

to, any recent decompression partial melting beneath Gakkel ridge. This is particularly well illustrated by the fact that the correlation between Os isotopes and bulk Al_2O_3 content would not exist if they had been affected by substantial partial melting beneath Gakkel ridge.

Effects of seawater contamination and melt refertilization

Two processes that might affect Os isotopes of abyssal peridotites are interactions with metasomatic fluids or melts and seawater contamination. The potential for seawater contamination to increase the radiogenic Os isotopes of abyssal peridotites has been debated for some time[23,31,32]. Ratios of $^{187}Os/^{188}Os$ from abyssal peridotites with clear signs of seafloor weathering have been suspected of seawater contamination and typically discounted from representing the depleted MORB mantle in previous studies[31]. The current sample set provides an excellent test of this hypothesis, as we have perfectly fresh, weathered and serpentinized samples (although the latter are from a different area). The $^{187}Os/^{188}Os$ ratios in the five weathered rims from dredge PS66-238 are slightly higher than in their corresponding interiors, with a maximum Os isotope ratio that is 2.3% higher in the harzburgite. Such a difference is significant compared with the external reproducibility of the analytical method (<0.4%), but small compared with the overall range in the data. The interiors of these samples are essentially very fresh, and thus are unlikely to show any seawater influence at all. Despite the very large difference in their alteration, both PS66-238 and D70 samples have similar ranges of $^{187}Os/^{188}Os$ ratios. Thus, seawater contamination has had a negligible effect on the Os isotopic compositions of these abyssal peridotites.

Melt refertilization is very common in abyssal peridotites from slow-spreading ridges[29]. Trace elements in clinopyroxenes indicate that Gakkel abyssal peridotites were refertilized by late-enriched melts after low-degree partial melting. Basaltic melts generally have more radiogenic $^{187}Os/^{188}Os$ ratios (0.125–0.23 compared with 0.12–0.13) but much lower Os contents (1–50 parts per 10^{12} (p.p.t.) compared with 1–4 parts per 10^9 (p.p.b.)) relative to abyssal peridotites[33]. Even the addition of 25% of an extreme hypothetical basaltic melt ($^{187}Os/^{188}Os = 0.23$ and [Os] = 50 p.p.t.) to an abyssal peridotite ($^{187}Os/^{188}Os = 0.127$ and [Os] = 3.4 p.p.b.) would result in only 0.4% change in the Os isotopic composition of a typical mantle peridotite. Thus, simple binary mixing with basaltic melt cannot significantly affect Os isotopic compositions in mantle rocks. On

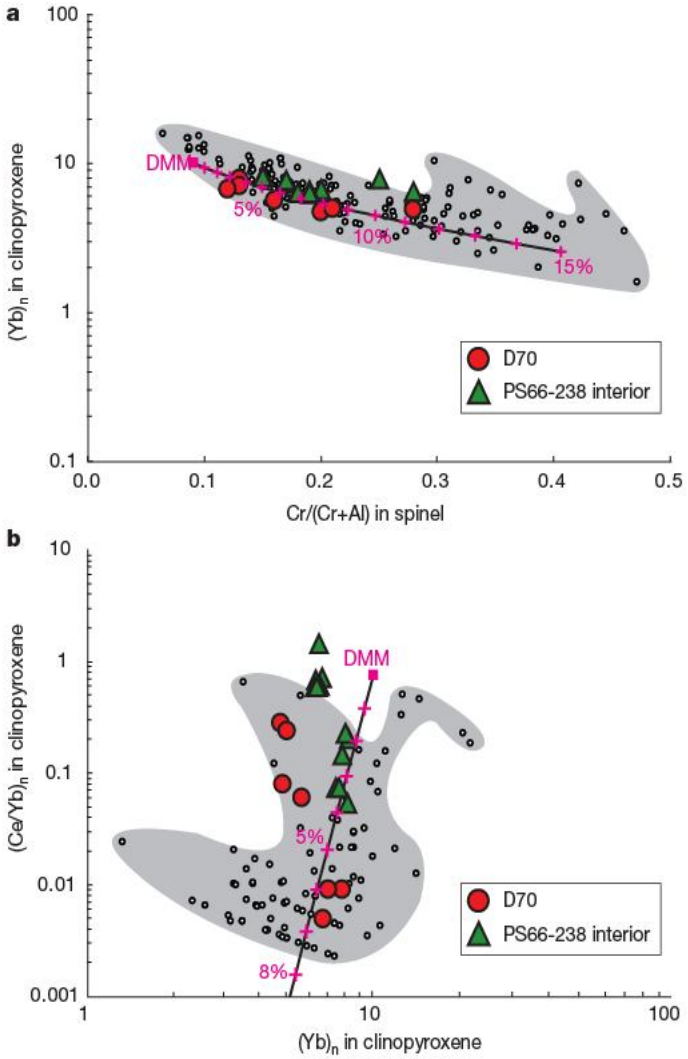

Figure 2 | Sample composition indicating partial melting and melt refertilization histories. **a**, Spinel Cr# versus $(Yb)_n$ in cpx (n, chondrite normalized). Low to moderate (5–12%) degrees of partial melting in Gakkel abyssal peridotites are estimated by Cr# (= Cr/(Cr+Al)) of the residual spinels, using a method described previously[21]. **b**, $(Ce/Yb)_n$ versus $(Yb)_n$ in cpx. Ce/Yb ratios in cpx from both D70 and PS66-238 deviate from the fractional partial melting trend, indicating the occurrence of melt refertilization. Chondrite normalized values are from ref. 47. The equation and parameters used in fractional partial melting modelling are from ref. 21 and DMM compositions are from ref. 8. The white small circles are abyssal peridotite data from the literature[21,48,49]

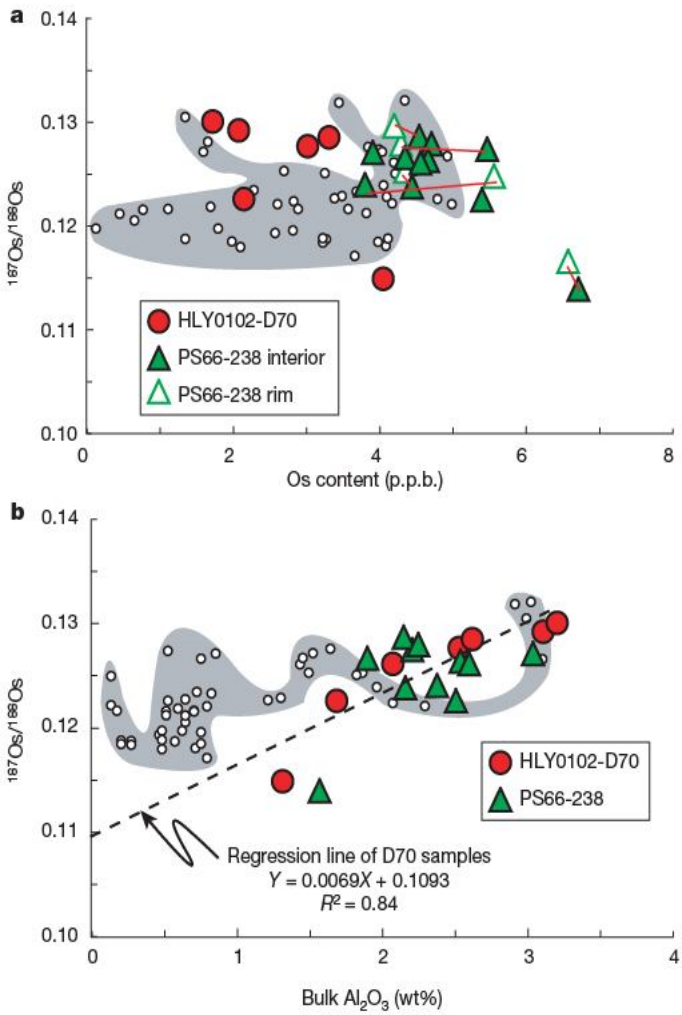

Figure 3 | Diagram of $^{187}Os/^{188}Os$ versus Os content and bulk Al_2O_3. **a**, Osmium contents of D70 samples are systematically lower than those of PS66-238 samples. Fresh interiors and their corresponding altered rims from the same sample are connected by the red lines. The altered rims generally have slightly higher $^{187}Os/^{188}Os$ ratios than their corresponding interiors, whereas there is no systematic variation of Os contents between rims and interiors. **b**, Both PS66-238 and D70 samples have similar ranges in both $^{187}Os/^{188}Os$ ratios and bulk Al_2O_3 contents. Bulk Al_2O_3 contents of D70 samples show good correlation with their $^{187}Os/^{188}Os$ ratios, which, however, is absent in PS66-238 samples. The white small circles are abyssal peridotite data from the literature[11,12,17,31,50].

the other hand, percolation of sulphide melts rather than basaltic melts would more effectively increase the $^{187}Os/^{188}Os$ ratios of the mantle peridotites[34]. For example, *in situ* study of sulphides by the laser ablation method has detected radiogenic $^{187}Os/^{188}Os$ in interstitial sulphides, which were explained as secondary sulphides and addition from melts[23]. However, the Al_2O_3–$^{187}Os/^{188}Os$ correlation in the D70 peridotites suggests that their Os isotopes have not been significantly affected by addition of either sulphides or basaltic melts. Otherwise, such a correlation would have been erased. The correlation between Re/Os and bulk Al_2O_3 content in the PS66-238 peridotites (Fig. 4) also indicates that the addition of pure sulphide melt did not occur in these samples.

Os isotopic heterogeneity in the oceanic mantle

Currently, there is no consensus on the Os isotopic composition of the DMM[31,35]. On the basis of the $^{187}Os/^{188}Os$ ratios of abyssal peridotites, it has been suggested that DMM has subchondritic $^{187}Os/^{188}Os$ ratios around 0.125, which implies a long-term Re depletion in DMM relative to primitive upper mantle (PUM)[31]. The continental crust does not balance the amount of Re depleted from the upper mantle, and therefore subducted oceanic crust, possibly stored in the lower mantle, has been proposed as a potential reservoir for such 'missing Re'[36]. Other studies, however, inferred DMM to have PUM-like $^{187}Os/^{188}Os$ ratios ≈ 0.129, indicating that little Re has been removed from the DMM as a whole[35]. This would imply that the refractory peridotites with unradiogenic $^{187}Os/^{188}Os$ ratios generally do not contribute to the MORB source region during melting in magmatically active or inactive ridge segments. Gakkel ridge is an extreme case, because the unradiogenic peridotites are refractory harzburgites and the overall degree of melting at the ridge is very low. They may thus preserve ancient signatures from the asthenosphere unaffected by a few per cent of partial melting at Gakkel ridge. On other ridges, however, pervasive wetting by melt results in compositional changes that largely obscure the relationship between the depleted and fertile portions of the Gakkel ridge mantle that we have sampled.

The wide range of $^{187}Os/^{188}Os$ ratios in Gakkel ridge abyssal peridotites reflects the coexistence (on the kilometre scale sampled by dredging) of fertile mantle domains with relatively radiogenic near-PUM $^{187}Os/^{188}Os$ ratios and refractory domains with unradiogenic $^{187}Os/^{188}Os$ ratios. The distribution of refractory and fertile upper mantle domains with different Os isotopic characteristics in the upwelling asthenosphere is, perhaps, also similar to a 'plum pudding' mantle[37]. Thus, a single Os isotopic composition of DMM cannot be established. The domains with unradiogenic $^{187}Os/^{188}Os$ ratios, represented by the harzburgites, have been subjected to ancient partial melting. The widely reported occurrences of unradiogenic $^{187}Os/^{188}Os$ ratios in both abyssal peridotites[11,12,17] and ophiolites[38–40] suggest that ancient partial melting events are ubiquitously recorded in the global asthenosphere[41]. On the other hand, PUM-like $^{187}Os/^{188}Os$ ratios in many lherzolites from ocean ridges suggest that either no significant Re has been removed in some fertile domains or they have been affected by components enriched in $^{187}Os/^{188}Os$.

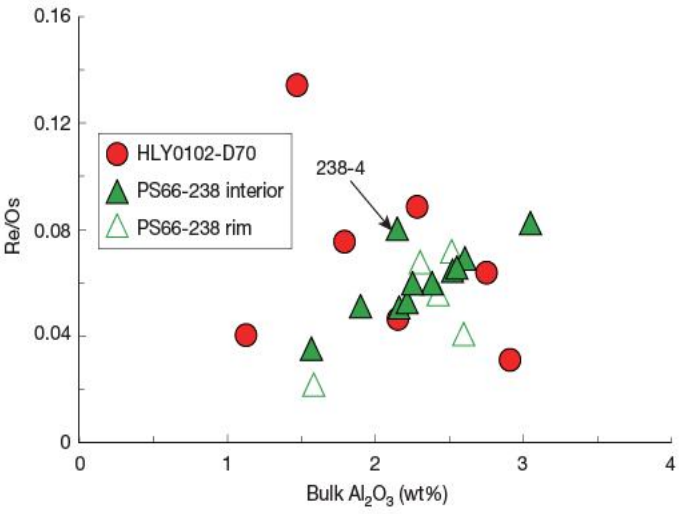

Figure 4 | Correlation between Re/Os ratios and bulk Al_2O_3 contents. All fresh interiors but one (238-4) show good positive correlation. The absence of such a relationship in both D70 samples and the altered rims of PS66-238 samples indicate the disturbance of Re during seawater alteration.

Long-time survival of ancient Os isotopic signals in asthenospheric mantle could also have substantial implications for intra-oceanic or continental mantle keel sources. A correlation between $^{187}Os/^{188}Os$ and Al_2O_3 in asthenospheric mantle, like in the D70 samples, could be produced by mixing of peridotites with different fertilities and ages, which have been preserved in the convecting asthenospheric mantle for a long time and delivered to mid-ocean ridges by mantle convection. Such a correlation, therefore, cannot be used to discriminate between subcontinental lithospheric mantle and asthenospheric mantle as has been previously proposed[25]. If mixtures of refractory and MORB-source (that is, relatively fertile) mantle characterize the asthenospheric mantle, young asthenospheric mantle could bear refractory domains with ancient Os isotopic signatures. The observation of relatively unradiogenic Os isotopic signatures in ophiolites (and mantle rocks generally) could simply represent the ambient heterogeneity of depleted harzburgitic domains in the oceanic mantle rather than definite evidence of delaminated subcontinental lithospheric mantle. In the meantime, the use of Os model ages of mantle xenoliths to constrain the ages of subcontinental lithospheric mantle should be approached with caution.

Is DMM the source of MORB?

Traditionally, the terms 'DMM' and 'MORB source' have been considered synonymous with each other, and with upwelling of a depleted asthenospheric mantle that may also contain enriched 'plums' or 'blobs'[3,7,8]. For incompatible elements, this assumption is valid. But it fails to take into account regions of mantle too refractory to take part in partial melting such as sections of Gakkel ridge. These refractory mantle domains have formed by ancient partial melting and been carried passively in the mantle until their emplacement on the sea floor. If observations from Gakkel ridge can be generalized to mantle beneath mid-ocean ridges as a whole, the source of MORB preferentially samples the more fertile portion of the total upwelling mantle, and not the coexisting domains of refractory material that do not melt and thus contribute little to MORB formation. Domains with different melting histories have been mechanically juxtaposed but not homogenized by mantle convection. The fertile mantle domains may or may not have a genetic relationship with neighbouring refractory domains[42].

If refractory domains are heterogeneously distributed in the asthenospheric mantle (Fig. 5), there are several interesting consequences for models of mantle dynamics. First, MORB does not sample the entire upwelling asthenosphere beneath the ocean ridges, but only the non-refractory domains, and thus MORB compositions do not necessarily constrain the nature or composition of the overall asthenosphere. This complicates the interpretation of geochemical data in much the same way as the hypothesis of enriched mantle veins[43,44], which our data do not address. Second, without knowing *a priori* which mantle domains participate in partial melting and to what extent, it is impossible to calculate a DMM (that is, asthenospheric mantle) Os isotopic composition relevant to the formation of MORB from observations in asthenospheric mantle peridotites. The same might also be true for other isotopic systems (for example, Rb–Sr, Sm–Nd, U–Pb and Lu–Hf). Third, observations of asthenospheric mantle composition based on observations on MORB that do not take refractory components into account will tend to overestimate the fertility of the upper mantle, particularly on ultraslow-spreading ridges.

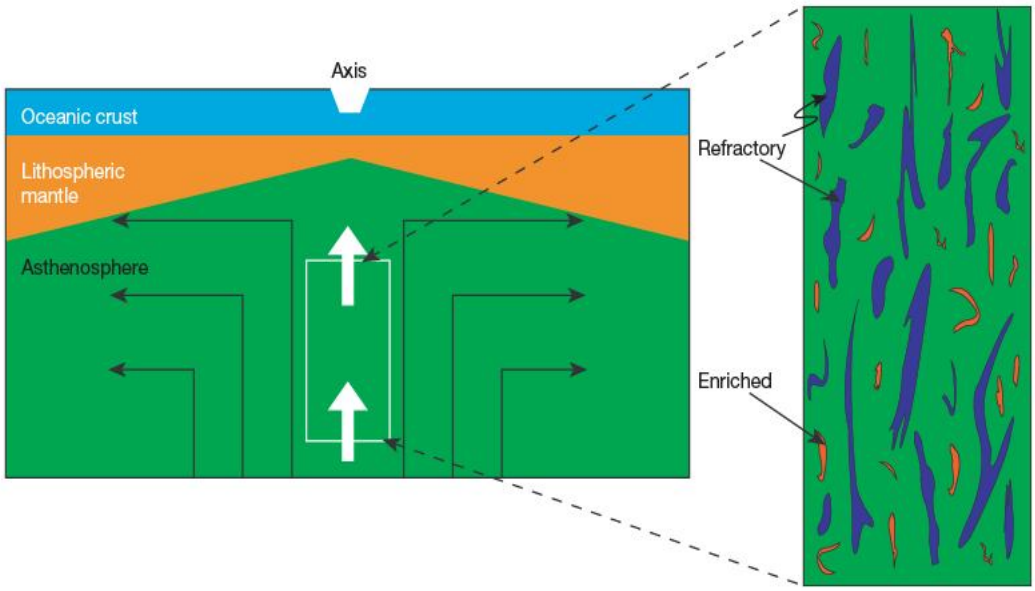

Figure 5 | Schematic diagram of the upwelling asthenospheric mantle beneath mid-ocean ridges. Both enriched and refractory mantle are distributed among the 'matrix' of normally depleted mantle. The enriched mantle and the normally depleted mantle can be sampled by E-MORB and N-MORB, respectively. However, it is hard to sample the refractory mantle with inherited ancient depletion signals because it contributes little to the genesis of MORB. Therefore, they are not a part of the 'MORB source' *sensu stricto*, although the latter has been generally taken to be synonymous with asthenospheric mantle.

METHODS SUMMARY

Major and trace elements. Major elements were analysed using X-ray fluorescence at the University of Mainz. Major elements of spinels were measured by JEOL JXA 8900RL electron microprobe at the University of Mainz, using an acceleration potential of 20 kV, a beam current of 12 nA and a spot size of 2 μm. Clinopyroxene trace-element data of D70 serpentinites were measured by Cameca ims-3f at the Max-Planck Institute of Chemistry (MPI), Mainz, using a method described previously[45]. We measured the trace elements of clinopyroxenes in the fresh PS66-238 samples by laser ablation/inductively coupled plasma mass spectrometry at MPI. Ablation was achieved with a New Wave UP-213 Nd:YAG laser system, using a pulse repetition rate of 10 Hz and crater diameter of 80 μm. Analyses were performed on a single collector sector-field Thermo Finnigan Element-2 mass spectrometer in pulse counting mode. We used ^{43}Ca as internal standard element. We used two internal standards (NIST-612 and KL2-G) and one external standard (GOR132-G), and their values are from GEOREM (http://georem.mpch-mainz.gwdg.de).

Re–Os isotopes. We determined Re–Os isotopes of the Gakkel abyssal peridotites at the Max-Planck Institute for Chemistry. The method has been described previously[46]. Samples of powder (2 g) were digested in capped quartz tubes together with a mixed Re–Os isotope tracer, 3 ml of 12 mol l^{-1} HCl and 7 ml 16 mol l^{-1} HNO_3 for 16 h in a high-pressure asher (HPA-S) at 100 bar and 300 °C. We separated Os from the solution by solvent extraction with bromine and purified it by micro-distillation. Afterwards, we extracted Re from the solution in anion exchange columns. We made Os isotope measurements by negative thermal ionization mass spectrometer (N-TIMS) on a Finnigan MAT 262 instrument. Total reagent blanks were <6 pg for Os and <20 pg for Re. The blank ^{187}Os/^{188}Os ratios are less than 0.3176. Repeat measurements of ^{187}Os/^{188}Os in a standard containing 35 pg Os yielded external precision of 0.1% (2δ). Reproducibility based on duplicate analyses of three samples was <0.2% for ^{187}Os/^{188}Os and <25% for Os concentration. The quality of the Os data from the Mainz laboratory was also confirmed in a comparative analytical study of serpentinite standard UB-N. Rhenium was measured on a 'Nu Plasma' multi-collector inductively coupled plasma mass spectrometer. The external precision (2δ) of standard solution (10–25 ng g^{-1}) measurements was 0.1–0.2% for Re.

Received 28 August 2007; accepted 8 January 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank the Captain and crew of PFS *Polarstern* expedition ARK XX/2 and the other participants in the AMORE 2001 expedition. This work was supported by the Max-Planck Institute for Chemistry and the Deutsche Forschungsgemeinschaft to J.E.S. We thank N. Groschopf and D. Kuzmin for help with electron microprobe analyses, K. P. Jochum, B. Stoll and K. Herwig for help with laser ablation ICPMS measurements, K. Zentel for help with the Os-PGE experiment, and S. Shirey for comments on the manuscript. C.-Z.L. acknowledges scholarships from both Chinese Academy of Sciences and Max-Planck Society.

Author Contributions C.-Z.L. and G.B. did the Os-PGE measurements on samples collected by two Arctic expeditions proposed and carried out by J.E.S., together with E.H., A.v.d.H. and A.B., who also provided preliminary and supporting data. A.W.H. provided intellectual and material support. All authors contributed to interpretation of the results and writing of the manuscript.

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ARTICLES

Thyrotrophin in the pars tuberalis triggers photoperiodic response

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Molecular mechanisms regulating animal seasonal breeding in response to changing photoperiod are not well understood. Rapid induction of gene expression of thyroid-hormone-activating enzyme (type 2 deiodinase, *DIO2*) in the mediobasal hypothalamus (MBH) of the Japanese quail (*Coturnix japonica*) is the earliest event yet recorded in the photoperiodic signal transduction pathway. Here we show cascades of gene expression in the quail MBH associated with the initiation of photoinduced secretion of luteinizing hormone. We identified two waves of gene expression. The first was initiated about 14 h after dawn of the first long day and included increased thyrotrophin (TSH) β-subunit expression in the pars tuberalis; the second occurred approximately 4 h later and included increased expression of *DIO2*. Intracerebroventricular (ICV) administration of TSH to short-day quail stimulated gonadal growth and expression of *DIO2* which was shown to be mediated through a TSH receptor–cyclic AMP (cAMP) signalling pathway. Increased TSH in the pars tuberalis therefore seems to trigger long-day photoinduced seasonal breeding.

Animals living outside the tropics use changes in photoperiod to adapt to seasonal changes in environment, but the molecular mechanisms underlying photoperiodic time measurement are not fully understood[1]. The Japanese quail is a robust model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. When quail are transferred from short to long days, plasma luteinizing hormone increases at the end of the first long day: this photoperiodic response is the core feature of the avian 'first day release model' of reproductive photoperiodism[2,3]. In birds, the components required for photoperiodic signal transduction are located in the mediobasal hypothalamus (MBH) and include a deep brain photoreceptor[4], a clock to measure daylength[5], and output pathways to regulate the secretion of gonadotrophin-releasing hormone (GnRH)[6,7]. Recently, we have reported that long-day-induced local activation of thyroid hormone metabolism in the quail MBH is an early event in photoperiodic signal transduction[8,9]. Under short-day conditions, expression of type 2 deiodinase (*DIO2*), which converts the prohormone thyroxine (T_4) to bioactive triiodothyronine (T_3), is maintained at a low level, whereas expression of type 3 deiodinase (*DIO3*), which metabolizes T_4 and T_3 to reverse (r)T_3 and T_2, respectively, is maintained at a high level. When quail are transferred from short to long days, rapid reciprocal switches in *DIO2* and *DIO3* expression occur at the end of the first long day, resulting in a local increase in T_3 concentration. This increase in MBH T_3 concentration precedes the first rise in the concentration of photoinduced plasma luteinizing hormone and is causally related. Administration of T_3 to short-day quail stimulates secretion of luteinizing hormone and testicular growth, whereas conversely, administration of a DIO2 inhibitor inhibits photoinduced testicular growth[8,10]. The question now is the identity of the photoperiodic transduction pathway regulating *DIO2* expression in the MBH.

To address this, we have dissected the molecular dynamics of gene expression regulating photoinduced thyroid hormone metabolism in the quail MBH during the first day of photoinduced luteinizing hormone secretion by using a chicken high-density oligonucleotide microarray. Quail and chicken are both galliforms with predicted high interspecific DNA sequence conservation. To test this prediction, we applied biotinylated chicken and quail genomic DNA to the array. Signals for 82.2% of the probes were statistically indistinguishable between the two species (Welch's *t*-test, Benjamini and Hochberg false discovery rate (FDR) multiple test, $P > 0.05$, $n = 3$) (Supplementary Fig. 1).

Genome-wide expression analysis

To study changes in gene expression during the first long day, eight-week-old male quail kept under short days (6/18 h light/dark cycle) for four weeks were transferred to long days (20/4 h light/dark cycle). Plasma samples and brains were collected from six birds every 4 h for three days during this transition. In addition, samples were collected every 2 h between 10 and 22 h after dawn of the first long day to cover the period during the initiation of the photoperiodic response when the most rapid changes in gene expression were predicted to occur[2,3]. The first increase in plasma luteinizing hormone was observed at 22 h after dawn of the first long day as previously reported[3,11] (Fig. 1a)

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(one-way analysis of variance (ANOVA), $F_{20,105} = 5.860$, $P < 0.0001$, Fisher's least significant difference (LSD) post-hoc test, $P < 0.05$, $n = 6$).

For each time point, biotinylated antisense RNAs (cRNAs) prepared from pooled MBH were hybridized to duplicate sets of arrays to minimize experimental error. Using the RMA algorithm and statistical cosine filters[12], we identified 77 cycling genes which were disqualified from consideration as long-day-induced genes (Fig. 1b and Supplementary Table 1). These genes included eight circadian clock genes (Supplementary Fig. 2). We next focused on genes showing 1.5-fold or more changes in expression during the first long day, and found two waves of expression initiated at around 14 h (peak time 16.46 h, Fig. 1a) and the other initiated at around 18 h after dawn (peak time 21.15 h, Fig. 1a) (Supplementary Table 2 and Supplementary Fig. 3) (Welch's one-way ANOVA, FDR $P < 0.01$).

The first wave comprised two genes encoding thyrotrophin-β (TSH-β) and eyes absent 3 (EYA3); the second wave comprised 11 genes including *DIO2* and *DIO3* which showed inversely related changes in expression (Supplementary Fig. 4). Using *in situ* hybridization, the expression of the two first-wave genes (*TSHB* and *EYA3*) was observed in the pars tuberalis of the pituitary gland, whereas expression of six of the second-wave genes including *DIO2* and *DIO3* was observed in the ependymal cells lining the ventro-lateral walls of third ventricle and in the adjacent infundibular nucleus (Fig. 1c).

We also noted rhythmic expression of the gene encoding common pituitary glycoprotein alpha subunit (CGA) in the pars tuberalis (Fig. 1c and Supplementary Fig. 2). The expression of this gene with that encoding the TSH-β suggests that the proteins encoded by these genes associate in the pars tuberalis to form TSH. This view is supported by the observation that TSH-β protein occurs in pars tuberalis cells (Fig. 2a). We therefore deduced that increased TSH in the pars tuberalis may be functionally significant for photoperiodic signal transduction. To test this hypothesis, we first determined whether TSH receptor (*TSHR*) gene expression occurs in the MBH.

TSH receptor in the MBH

Strong expression of *TSHR* was observed in the ependymal cells and pars tuberalis; weak expression was observed in the infundibular nucleus (Fig. 2b). *TSHR* expression in the pars tuberalis was detected at 6 and 22 h after dawn of the first long day but not at 14 h, whereas it occurred in the ependymal cells all the times examined ($n = 2$). To verify these results, we further performed a ^{125}I-labelled TSH binding assay. We first demonstrated specific binding of ^{125}I-labelled TSH in the thyroid gland of quail as a positive control (Fig. 2c). Specificity of the binding assay was also confirmed by radioreceptor assay (Supplementary Fig. 5). We then observed specific binding of ^{125}I-labelled TSH in the ependymal cells, infundibular nucleus and the pars tuberalis. This observation is consistent with expression sites of *TSHR* mRNA at these loci. Although TSH binding in the ependymal cells was observed at all the times examined, that in the pars tuberalis was undetectable at time 22 h (Fig. 2d). Because the median eminence is one of the circumventricular organs and is outside the blood–brain barrier[13], long-day-induced TSH in the pars tuberalis has the potential to enter the brain to interact with TSHR in the ependymal cells and infundibular nucleus. We therefore predicted that the

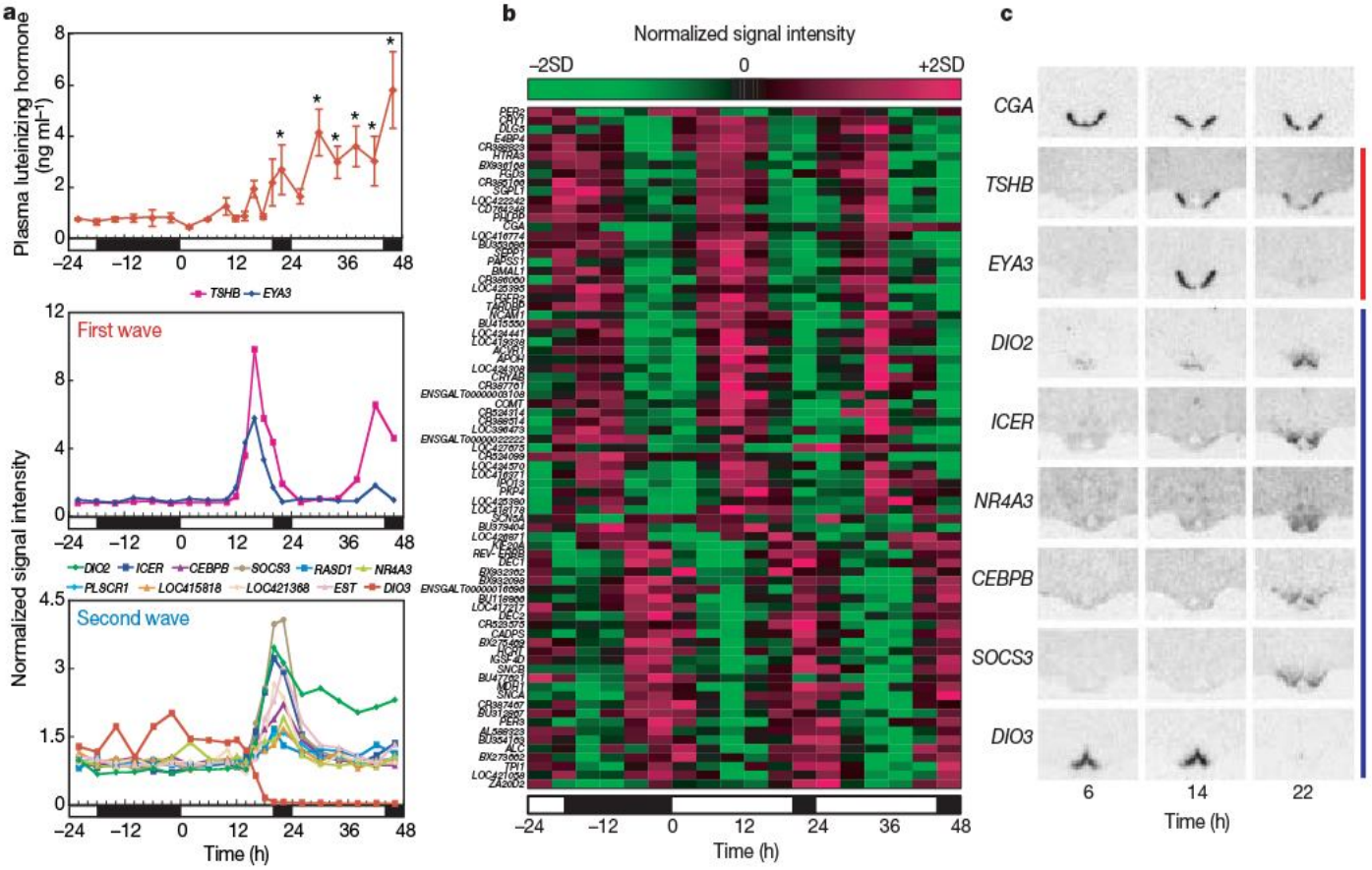

Figure 1 | Plasma luteinizing hormone and genome-wide analysis of genes expressed in the quail MBH during the first day of photostimulation (time 0 h is dawn of the first long day). **a**, Changes in plasma luteinizing hormone (mean ± s.e.m., $n = 6$, $^*P < 0.05$ versus the value at time −22 h); and timing of first- and second-wave gene expression. Data are normalized such that the median signal strength for each gene over all time points was 1.0. The average signal strength at each point was then displayed as a ratio relative to the median signal strength of that gene. **b**, Organization of 77 genes showing 24 h rhythmic changes in expression. Data are normalized such that the mean and s.d. of log expression values over all time points for each gene are 0 and 1, respectively. Italic script, gene identities and accession numbers. **c**, Spatio-temporal expression of common glycoprotein hormone subunit (*CGA*), first-wave (red bar) and second-wave genes (blue bar). Expression of *CGA* and first-wave genes was observed in the pars tuberalis, whereas that of second-wave genes was observed in the ependymal cells and the infundibular nucleus.

photoinduced increase in pars tuberalis TSH may function to stimulate the expression of *DIO2* and possibly other second-wave genes.

TSH regulation of *DIO2* gene

To test this prediction, a range of doses (0.01, 0.1, 1.0 mIU) of bovine TSH[14] dissolved in 10 µl saline was administered 16 h after dawn to short-day quail to correspond with the time that *TSHB* expression is at its highest in the pars tuberalis after dawn of a first long day. Brains were collected 4 h after the injection when the induction of second-wave genes was predicted to be maximal. As shown in Fig. 3a, b, intracerebroventricular (ICV) TSH injection induced the expression of the *DIO2* and three other second-wave genes in a dose-dependent manner (one-way ANOVA, Fisher's LSD post-hoc test, $P < 0.05$, $n = 3$–6). Induction of these genes was observed in the dorsal and ventrolateral ependymal cells and in the infundibular nucleus (Figs 3a and 5a) and was more prominent in the ependymal cells than in photostimulated birds (Fig. 1c and Supplementary Fig. 6). This is likely to be a consequence of the periventricular ependymal cells being more assessable to ICV TSH than to TSH originating from the pars tuberalis. We further confirmed this physiological effect of TSH by ICV injection of TSH-β antibody to long-day quail. Anti-chicken/quail TSH-β IgG[15] or pre-immune serum IgG (1 µg per 10 µl) was administered every 2 h (ref. 16) from 12 h to 18 h after dawn of the first long day, and brains were collected 2 h after the last injection. As shown in Fig. 3c, d, anti-TSH-β IgG injections suppressed the expression of the four second-wave genes, including *DIO2*, shown to be induced by ICV TSH.

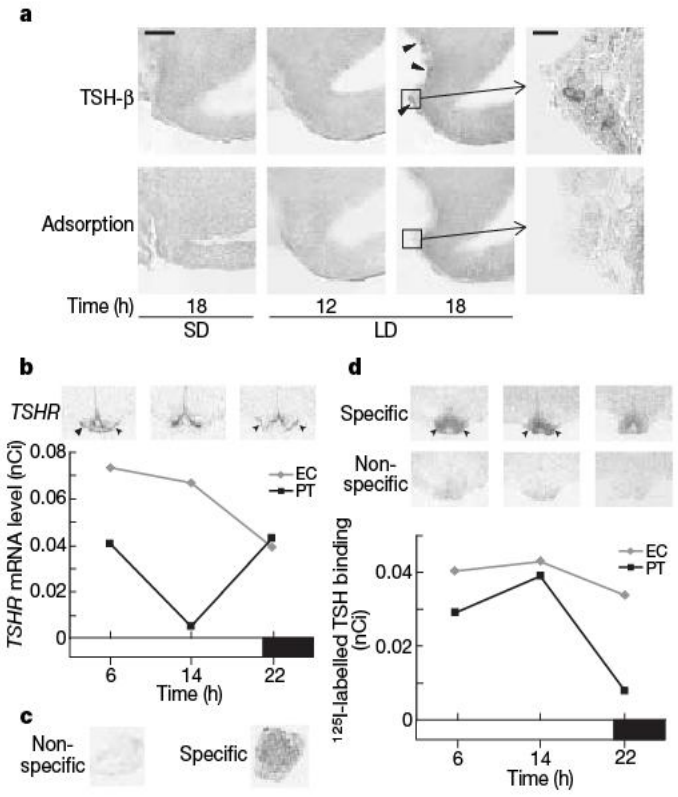

Figure 2 | Localization of TSH-β and TSHR in the pars tuberalis and MBH. **a**, Positive immunolabelling for TSH-β in the pars tuberalis (arrowhead) induced by the long-day stimulus was eliminated by pre-adsorption of the anti-TSH-β antibody with the synthetic TSH-β peptide sequence used to produce the antibody. Scale bars: left, 100 µm; right, 10 µm. SD, short day; LD, long day. **b**, Expression of *TSHR* mRNA in the ependymal cells was observed at all the times examined, whereas that in the pars tuberalis (arrowhead) was not observed at time 14 h ($n = 2$). **c**, **d**, Binding of ^{125}I-labelled TSH to quail thyroid gland (**c**) and the MBH (**d**). Although TSH binding in the ependymal cells and the infundibular nucleus was observed at all the times examined, that in the pars tuberalis was not observed at time 22 h ($n = 2$).

Involvement of cyclic AMP signalling pathway

To further address the mechanism through which TSH might regulate the expression of *DIO2* and three other second-wave genes we first determined the transcriptional start sites using the oligo-capping method[17] and mapped them to quail and chicken genome sequences (Fig. 4a, Supplementary Fig. 7a). It is reported that the expression of *DIO2* in human thyroid gland is regulated through a TSHR-Gsα-cAMP regulatory cascade[18]. We found several putative cAMP responsive elements (CREs) in the 1.5 kilobase (kb) 5′ upstream regions of *DIO2*, in quail and chicken (Fig. 4a) and in the three other second-wave genes (Supplementary Fig. 7a). Conservation of CREs between the two species suggests the functional significance of this element (Fig. 4b and Supplementary Fig. 7b). To validate whether these CREs are involved in the regulation of *DIO2* gene by TSH, we analysed the promoter activity of the *DIO2* gene transfected into the 293 cell line. TSH administration induced expression of *DIO2* reporter activity in a dose-dependent manner only when *TSHR* was co-transfected (Supplementary Fig. 8). However, when CREs were mutated, induction by TSH was not observed (Fig. 4c). These results demonstrate that induction of the *DIO2* gene by TSH involves a cAMP signalling pathway through TSHR.

Photoperiodically regulated output genes

We next performed a microarray analysis on quail kept under short- and long-day conditions for two weeks to assess the chronic effects of photostimulation on MBH gene expression. MBH samples were collected from six birds every 4 h during a 24 h lighting cycle. This analysis identified 183 differentially expressed genes (Welch's two way ANOVA, FDR $P < 0.05$) (Supplementary Fig. 9a, b and Supplementary Table 3). Among these genes, 124 were upregulated and 59 were downregulated under long-day conditions (Supplementary

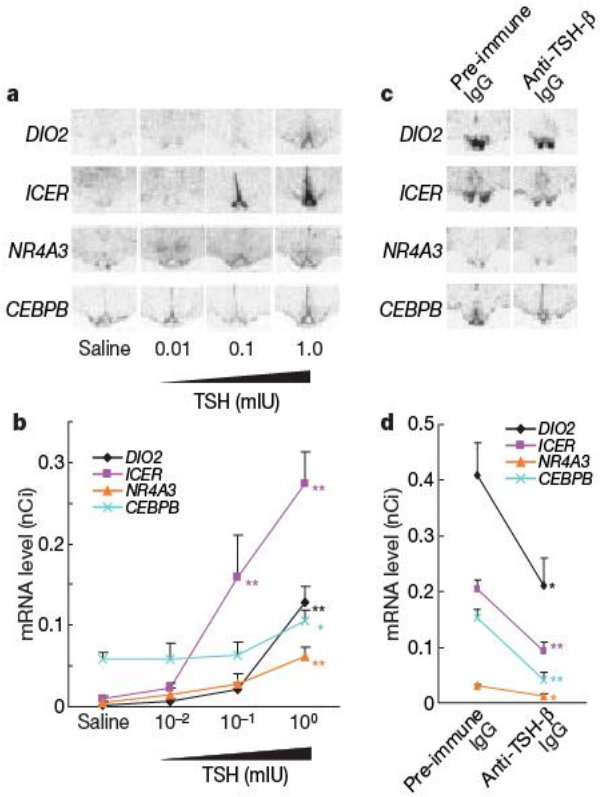

Figure 3 | Induction of the expression of *DIO2* and three other second-wave genes by ICV injection of TSH and inhibition by ICV injection of anti-TSH-β IgG. ICV injection of TSH (**a**, **b**) and anti-TSH-β IgG (**c**, **d**). Representative autoradiograms (**a**, **c**) and densitometric quantification (**b**, **d**) showing the effect of ICV injections of TSH/anti-TSH-β (**b**, $^*P < 0.05$, $^{**}P < 0.01$, ANOVA, Fisher's LSD post-hoc test, $n = 3$–6; **d**, $^*P < 0.05$, $^{**}P < 0.01$, *t*-test, mean + s.e.m., $n = 3$).

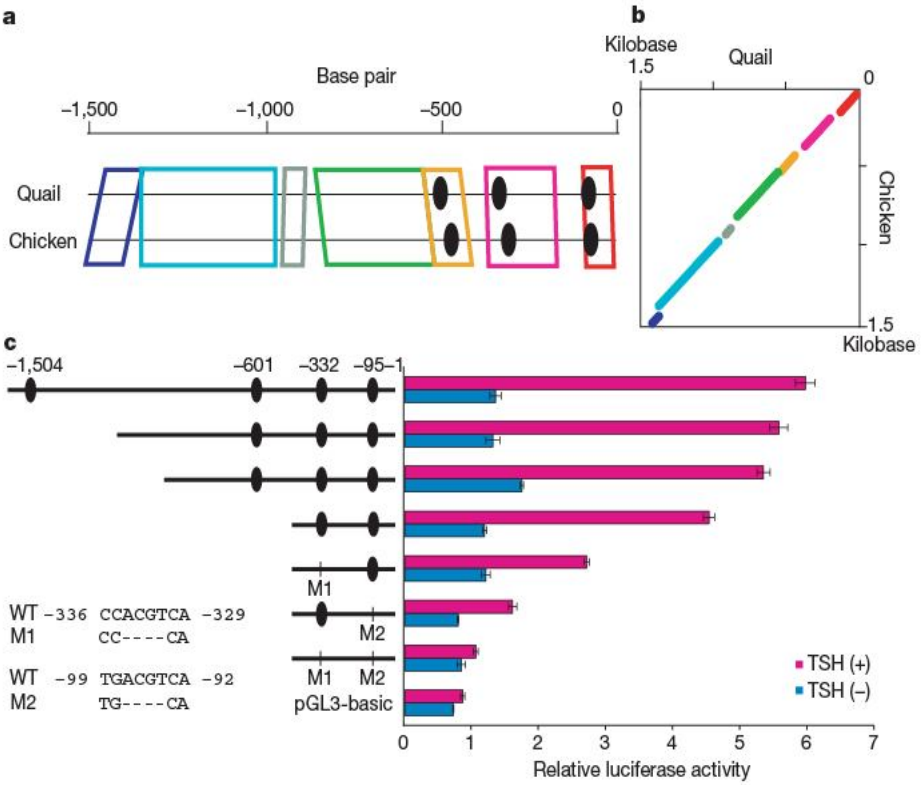

Figure 4 | Involvement of a cAMP signalling pathway in TSH induction of *DIO2* gene expression. **a**, Comparison of quail and chicken *DIO2* gene 5′ upstream sequences. Conserved segments are boxed. Filled ovals, putative CRE sites. **b**, Dot plot analysis of 5′ upstream regions using BLASTN, with greater than 85% identity. **c**, Promoter activity of quail *DIO2*. Wild-type and deletion/mutant reporters fused to the luciferase gene were assayed for their activities in response to TSH. Each value represents the mean ± s.e.m. of three replicates for a single assay.

Fig. 9a, b). We found long-day-induced expression of *DIO2* and reduced expression of *DIO3*, as previously reported[8,9] (Supplementary Fig. 10). *In situ* analysis of genes with known functions and showing differences in expression between long and short days, in the MBH, other than *DIO2* and *DIO3*, confirmed the microarray analysis (Supplementary Fig. 9c). In addition, we found a set of genes encoding various hormones and hormone receptors, which included *TSHB* and *CGA* (Supplementary Fig. 10 and Supplementary Table 3). Because high expression of *TSHB* and *CGA* was observed under chronic long-day conditions, we deduced that increased pars tuberalis TSH may not only play a role in initiating photoinduced secretion of luteinizing hormone, but may also be necessary to maintain the expression of other genes required to support a full reproductive response. We therefore investigated this possibility by prolonged ICV infusion of TSH (1.2 mIU per day) in short-day quail between 8 and 10 weeks of age. This treatment simulated MBH *DIO2* expression and gonadal development (Fig. 5).

Discussion

We have used the 'first day release model' of photoinduced luteinizing hormone release in quail to dissect the temporal pattern of changes in gene expression in the MBH associated with the initiation of photoinduced reproductive function. We found 77 genes that displayed a temporal pattern of expression under short and long days that would be expected of clock genes or clock-driven genes. Because most cycling genes are tissue specific[12,19], future analyses of the relations between the functions of these genes are likely to reveal further details of the molecular basis of the photoperiodic response. Our most important observation was the photoinduction of a first wave of gene expression initiated about 14 h after dawn of the first long day, comprising *TSHB* and *EYA3* in the pars tuberalis. These changes in gene expression are the earliest yet reported, to our knowledge, for the photoperiodic signal transduction pathway. This was followed approximately 4 h later by a second wave of gene expression in the ependymal cells and infundibular nucleus and included an increase in *DIO2*, a key element in the photoperiodic signal transduction pathway[8]. EYA3 is a transcriptional co-activator involved in the development of the eye and forms a nuclear complex with SIX (sine oculis) DNA-binding homeodomain factor and DACH (dachshund) nuclear cofactors[20]; we considered the possibility that the photoinduction of *EYA3* may induce expression of second-wave genes. However, this is unlikely because if *EYA3* is involved in second-wave gene expression it would need to be co-localized with these genes to exert its function. In the present study, *EYA3* was expressed in the pars tuberalis whereas second-wave genes were expressed in the ependymal cells and infundibular nucleus. Several *SIX* genes were observed in the pars tuberalis (Supplementary Fig. 11), suggesting that these may interact with long-day-induced *EYA3* to regulate the expression of genes, the identity of which remains to be established. We therefore focused on the possibility that *TSHB* in the pars tuberalis might be involved in the initiation of *DIO2* and the expression of other second-wave genes. Among the various cycling genes, we found rhythmic expression of *CGA*, and the peak of *CGA* preceded that of

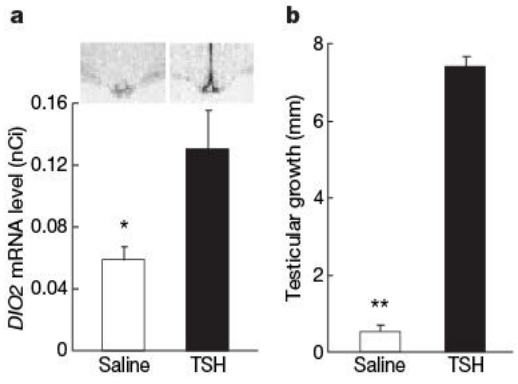

Figure 5 | Effect of chronic ICV infusion of TSH on MBH *DIO2* expression and testicular growth under short-day conditions. **a**, MBH *DIO2* expression. **b**, Testicular growth. (*$P < 0.05$, **$P < 0.01$, *t*-test, mean + s.e.m. $n = 5$).

long-day-induced *TSHB*. The biological activity of TSH requires a non-covalent association of CGA and TSH-β[21]. Thus it appears that the cycling CGA is available for dimerization with long-day-induced TSH-β to form bioactive TSH. It is also of note that translation of TSH occurs at least within 20 min[21]. In addition, unlike luteinizing hormone, dimerization of TSH-β and CGA, and secretion of TSH is very rapid and efficient, with a value of $t_{1/2}$ for the intracellular disappearance (that is secretion) of about 1 h (ref. 21). The expression of *CGA* and *TSHB* in the pars tuberalis therefore indicates that this is a source of biologically active TSH which may be transported into the third ventricle, possibly through tanycytes which abut the pars tuberalis[22]. The target site for pars tuberalis TSH was suggested by the presence of *TSHR* gene expression in the ependymal cells, infundibular nucleus and the pars tuberalis. This observation was supported by a ^{125}I-labelled TSH binding assay which showed specific TSH binding in these loci. Observations on the effects of ICV injection of TSH and anti-TSH-β IgG on *DIO2* and three other second-wave genes demonstrated that TSH triggers the expression of these genes in the ependymal cells. Furthermore, promoter analysis indicated that the induction of *DIO2* is likely to be mediated by the cAMP-signalling pathway. This observation is consistent with the action of TSH on human thyroid gland and rat brown adipose tissue, where *DIO2* expression is regulated by a TSHR–cAMP mediated mechanism[18,23]. Our study revealed a similar TSHR–cAMP mediated mechanism in the quail MBH. In addition to the acute effect of ICV TSH, chronic administration of TSH maintained increased *DIO2* expression and induced testicular growth under short-day conditions. This suggests that elevated TSH in the pars tuberalis may be required to maintain photoinduced reproductive function.

Although it is known that several species become photoperiodically blind after thyroidectomy, quail can respond to photoperiod even after thyroidectomy[1]. Recently, we have reported the involvement of TGF-α in the photoperiodism, and that the TGF-α signalling pathway is not dependent on thyroid hormone activity[24]. Interestingly, the similarity in expression profile between *DIO2* and *TGF-α* suggested that these two genes share the same transcriptional regulation. Although we failed to detect *TGF-α* gene expression in the present microarray analysis, we found TSH induced expression of *TGF-α* (Supplementary Fig. 12a, b). It is, therefore, possible that pars tuberalis TSH may signal photoperiodic information through both DIO2 and TGF-α. The magnitude of testicular growth induced by ICV TSH administration in short-day quail was indistinguishable from that of intact birds kept under long-day conditions (Supplementary Fig. 12c). This suggests that TSH is important not only for triggering photoperiodic responses, but also for the maintaining photoperiodically induced reproductive neuroendocrine function.

Since the discovery of dense melatonin receptors in the pars tuberalis in most mammalian species, but not birds, the pars tuberalis in mammals is considered to be involved in the transmission of photoperiodic stimuli to endocrine outputs through melatonin[25,26]. Further, the thyrotrophe cell type in the mammalian pars tuberalis expresses a high density of melatonin receptors and may regulate seasonal prolactin secretion[27]. However, unlike mammals, there is no evidence that circulating melatonin plays a role in photoperiodic transduction in birds[28]. Consequently, the mechanism transducing photoperiodic information to the avian pars tuberalis remains to be discovered. However, pars tuberalis TSH may be an evolutionarily conserved element of a photoperiodic signal transduction pathway in birds and mammals. This view is consistent with an earlier finding in sheep that expression of TSH-β in the pars tuberalis is not regulated by classical thyrotrophe receptors and their intracellular pathways, but through a novel, photoperiod-dependent mechanism[29].

Our view that photoinduced pars tuberalis TSH in the quail enters the cerebrospinal fluid to induce a photoperiodic response is consistent with the finding in the hamster that photoperiod-dependent changes in TSH-like immunoreactivity occur in the pars tuberalis[30] whereas TSH is found in the cerebrospinal fluid and central nervous system (CNS) of mammals[31,32]. The expression of *TSHR* has been reported in the mammalian brain[33,34], but no function has been proposed in relation to the control of photoperiodic responsiveness. In the present study, we show that long-day-induced TSH in the pars tuberalis triggers the expression of *DIO2* in the ependymal cells (Supplementary Fig. 13). To our knowledge, this is the first demonstration of the likely functional significance of pars-tuberalis-derived TSH in the CNS. Recently, it has been proposed that the pars tuberalis in sheep may be the circannual pacemaker for seasonal prolactin secretion[35]. Thus, the pars tuberalis appears to be the locus for the control of seasonality both in birds and other vertebrates.

In conclusion, one of the most important questions in photoperiodism is the identity of the molecular basis of the mechanism underlying the photoinducible phase that in the quail occurs 12–16 h after dawn[2]. Because photoinduction of *TSHB* expression was observed from about 12 h after dawn of the first long day, increased *TSHB* expression in the pars tuberalis may be the key molecular event defining the onset of the photoinducible phase. Our study presents the first comprehensive analysis of changes in hypothalamic gene expression likely to be involved in the regulation of the long-day reproductive photoperiodic response, and identifies pars tuberalis TSH as a key factor controlling photoperiodic signal transduction. The identification of a key role for *TSHB* expression in the pars tuberalis in reproductive photoperiodic time measurement marks a major advance in our knowledge of molecular mechanisms controlling seasonal breeding.

METHODS SUMMARY

Animals. We used Japanese quail (*C. japonica*) obtained from a local dealer and chicken (*Gallus domesticus*) (WL-G) kept in our colony. Because female birds have ZW chromosomes, they were used for genomic DNA analysis. In all other experiments, male quail were used. The present study was approved by the Committee on Animal Experiments of the Graduate School of Bioagricultural Sciences, Nagoya University.

Microarray experiments. We used Affymetrix Chicken Genome Array. This array contains over 38,000 probe sets representing 32,773 transcripts. Genomic DNA individually extracted from liver using DNeasy tissue kit (QIAGEN) was labelled by BioPrime DNA labelling system (Invitrogen). The MBH was punched out (2.5 mm diameter) from 3 mm quail brain slices generated using a mouse brain matrix. Total RNA was prepared from two pools of three MBH at each time point to duplicate our observations on two arrays, using Trizol reagent (Invitrogen); cDNA synthesis and cRNA labelling reactions were performed with One-Cycle Target Labelling and Control Reagents Kit (Affymetrix). Hybridization, wash and stain protocols and scanning were performed using standard Affymetrix protocols. Data were analysed by using GeneSpring GX7.3 software (Agilent Technologies).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 22 July 2007; accepted 25 January 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank T. Kondo and the Nagoya University Radioisotope Centre for use of facilities. We also thank J. A. Proudman and A. F. Parlow for providing the chicken luteinizing hormone radioimmunoassay kit and the bovine TSH, respectively. This work was done as a part of the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) and, in part, by Grant-in-Aid for Scientific Research (S), (B), Grant-in-Aid for Young Scientists (S), Grant-in-Aid for JSPS Fellows from the Japanese Society for the Promotion of Science, and a grant of the Genome Network Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Author Contributions T.Yo. conceived and directed the work. N.N., H.O., T.Ya., T.A., T.T., K.H., S.Y., Y.K., S.K., Y.U. and T.Yo. performed the microarray analysis and *in situ* hybridization. N.N., T.K., H.R.U. and T.Yo. analysed the microarray data. N.N. performed the quantitative PCR and promoter assay. M.I. and P.J.S. determined the luteinizing hormone assay. H.O. and M.I. performed the ^{125}I-labelled TSH binding assay. T.Ya. and A.I. performed the immunocytochemistry. T.Ya., T.A. and A.I. examined the ICV injection and infusion. N.N., H.O., Y.S. and S.S. determined transcriptional start sites and genomic DNA sequences. T.Ni. cloned EYA, SIX and DACH family. M.M., T.Na. and S.E. provided laboratory facilities and new materials. All authors discussed the results and commented on the manuscript. T.Yo. and P.J.S. wrote the paper.

Author Information The microarray data and DNA sequence information have been deposited in NCBI Gene Expression Omnibus (GEO) (GSE8016–GSE8018) and DDBJ/EMBL/GenBank (AB307676–AB307681), respectively. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to T.Yo. (takashiy@agr.nagoya-u.ac.jp).

ARTICLES

Translational control of the innate immune response through IRF-7

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Transcriptional activation of cytokines, such as type-I interferons (interferon (IFN)-α and IFN-β), constitutes the first line of antiviral defence. Here we show that translational control is critical for induction of type-I IFN production. In mouse embryonic fibroblasts lacking the translational repressors 4E-BP1 and 4E-BP2, the threshold for eliciting type-I IFN production is lowered. Consequently, replication of encephalomyocarditis virus, vesicular stomatitis virus, influenza virus and Sindbis virus is markedly suppressed. Furthermore, mice with both *4E-BP1* and *4E-BP2* genes (also known as *Eif4ebp1* and *Eif4ebp2*, respectively) knocked out are resistant to vesicular stomatitis virus infection, and this correlates with an enhanced type-I IFN production in plasmacytoid dendritic cells and the expression of IFN-regulated genes in the lungs. The enhanced type-I IFN response in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ double knockout mouse embryonic fibroblasts is caused by upregulation of interferon regulatory factor 7 (*Irf7*) messenger RNA translation. These findings highlight the role of 4E-BPs as negative regulators of type-I IFN production, via translational repression of *Irf7* mRNA.

Virus infection activates a subset of genes encoding cytokines and other antiviral proteins that trigger first the innate immune response and subsequently the adaptive immune response. Type-I interferons (IFN-α and IFN-β) are widely expressed cytokines that constitute the first line of defence against virus infections[1–4]. Although transcriptional control of IFN gene expression has a major role in the activation of the innate immune response, it is not known whether translational control is important for this process. Translational control of gene expression provides the cell with a rapid response to external and internal triggers or cues, without invoking the slower nuclear pathways for mRNA synthesis and transport. In eukaryotes, translational control mostly occurs at the rate-limiting initiation step, during which the small 40S ribosomal subunit is recruited to the mRNA[5]. Ribosome recruitment is facilitated by the 5′-cap structure (m^7GpppN, where N is any nucleotide) present on all nuclear transcribed eukaryotic mRNAs[6]. The cap structure is recognized by eukaryotic initiation factor 4F (eIF4F)[7], which consists of eIF4E, the cap-binding subunit[8], eIF4A, a bidirectional RNA helicase[9], and eIF4GI or eIF4GII, scaffolding proteins that bind directly to eIF4E and eIF4A and bridge the mRNA to the ribosome through interaction with eIF3 (ref. 10).

eIF4F complex assembly is inhibited by the 4E-BP translational repressors[7]. Mammals contain three highly related 4E-BPs that compete with eIF4G for a shared binding site on the convex dorsal surface of eIF4E[11,12]. mTOR-mediated phosphorylation of 4E-BP1 (the best characterized 4E-BP) stimulates translation by dissociating 4E-BPs from eIF4E. Hypophosphorylated 4E-BP1 binds to eIF4E with high affinity, whereas increased phosphorylation decreases its affinity for eIF4E[13].

Because viruses have evolved elaborate strategies to usurp the host translation machinery[14], we wished to study the effect of 4E-BPs on virus infections. To this end, we used mouse embryonic fibroblasts (MEFs) and mice deficient in 4E-BP1 and 4E-BP2.

Virus infection is suppressed in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs

To study the role of 4E-BPs in the cell's response to virus infections *ex vivo*, MEFs derived from *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ double knockout and wild-type mice were used[15]. The *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ double knockout MEFs lack all three 4E-BPs, because 4E-BP3 is not expressed in MEFs[16]. MEFs were infected with vesicular stomatitis virus (VSV) at a multiplicity of infection (MOI) of 0.5 plaque-forming units (PFU) per cell and viral protein synthesis was analysed by pulse labelling with [^{35}S]methionine at various times after infection. In wild-type MEFs, synthesis of VSV proteins was first detected at 4 h after infection (Fig. 1a). Notably, in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs, no viral proteins were detected at this, or even later, time points (Fig. 1a). Western blot analysis over the time course of infection demonstrated a robust expression of VSV proteins in wild-type MEFs, but not in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs (Fig. 1b). A VSV-induced cytopathic effect at 10 h after infection was observed only in wild-type MEFs (Fig. 1c). The lack of 4E-BPs resulted in reduced (~700-fold) virus titres (Fig. 1d). These data demonstrate that removing 4E-BPs abrogates VSV propagation. At a higher MOI (5 PFU per cell), the difference in the kinetics of VSV replication in wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs was less pronounced. In wild-type MEFs, VSV proteins were first detected as early as 2 h after infection, as compared to 4 h after infection in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs (Supplementary Fig. 1A). The VSV-induced shut off of host translation in wild-type MEFs occurred at 5 h after infection, whereas in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs, the reduction of cellular translation was barely detectable at 6 h after infection. In agreement with these data, the lack of 4E-BPs resulted in a decrease in infectious virus production (Supplementary Fig. 1B).

To confirm that the VSV-resistant phenotype observed in the *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs is due to the absence of 4E-BPs, and not to some unintended effect of the gene-targeting manipulation, we introduced 4E-BP1 together with 4E-BP2 into *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$

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MEFs. Expression of 4E-BP1 and 4E-BP2, but not empty vector, restored the susceptibility of the *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs to VSV infection (Supplementary Fig. 2). These data demonstrate that the virus-resistant phenotype of *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs is directly associated with the lack of 4E-BPs.

Next, we investigated whether *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs are resistant to infection by other viruses. Wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs were infected with positive and negative strand RNA viruses, such as Sindbis virus (alphavirus, positive strand), encephalomyocarditis virus (EMCV; picornavirus, positive strand) and influenza virus (orthomyxovirus, negative strand). Similar to VSV (rhabdovirus, negative strand), protein synthesis of Sindbis virus (Supplementary Fig. 3A), EMCV (Supplementary Fig. 3B) and influenza virus (Supplementary Fig. 3C) was severely impaired in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$, as compared to wild-type, MEFs. Accordingly, a marked reduction (>3,000-fold) in virus titres was observed for all three viruses in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs (Supplementary Fig. 3D). Taken together, these data demonstrate that the lack of 4E-BPs markedly impairs the replication of a broad spectrum of viruses.

Upregulation of type-I IFN response in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs

How do the 4E-BPs affect the replication of disparate viruses? A simple explanation would be that the threshold for eliciting type-I IFN production is lowered in MEFs lacking 4E-BPs. To test this hypothesis, wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs were treated with poly(I:C), a synthetic double-stranded (ds)RNA that is a potent inducer of type-I IFN. The production of IFN was determined by assessing the inhibition of the VSV-induced cytopathic effect. Six hours after treatment with poly(I:C), culture medium from wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs was collected and added to wild-type MEFs (Fig. 2a). After overnight incubation, MEFs were infected with VSV (MOI of 0.1 PFU per cell) and virus yield was determined by a plaque assay. Culture medium from wild-type MEFs treated with a low concentration of poly(I:C) (0.1 μg ml^{-1}) failed to protect cells from VSV infection (Fig. 2b). In contrast, no cytopathic effect was observed and virus production was significantly reduced (~200-fold) in wild-type MEFs incubated with the culture medium from *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs. Consistent with the notion that the enhanced resistance of the *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs to virus infection is due to increased type-I IFN production, incubation with a neutralizing antibody against IFN-β rescued the VSV-resistant phenotype (Supplementary Fig. 4A).

Expression of IFN-α (*Ifna*) and IFN-β (*Ifnb*) mRNAs was more responsive to treatment with either 0.1 μg ml^{-1} (Fig. 2c) or 1 μg ml^{-1} (Supplementary Fig. 4B) of poly(I:C) in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs compared with wild-type MEFs, as determined by reverse transcriptase polymerase chain reaction (RT–PCR). Moreover, the induction of *Ifna* and *Ifnb* mRNA synthesis after VSV infection was greater in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ than in wild-type MEFs (Supplementary Fig. 4C). Consistent with these data, poly(I:C) treatment of *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs, but not wild-type MEFs, elicited a robust production of IFN-α (Fig. 2d and Supplementary Fig. 5C) and IFN-β (Supplementary Fig. 5A, B), as determined by enzyme-linked immunosorbent assay (ELISA). Collectively, these data show that the lack of 4E-BPs results in enhanced type-I IFN production.

Double knockout mice are protected against VSV infection

To determine whether the virus-resistant phenotype of *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs is recapitulated *in vivo*, wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice were infected intranasally with VSV (5×10^7 PFU). We chose VSV because its replication is exquisitely sensitive to inhibition by type-1 IFN[17]. By day 6 after infection, 80% of

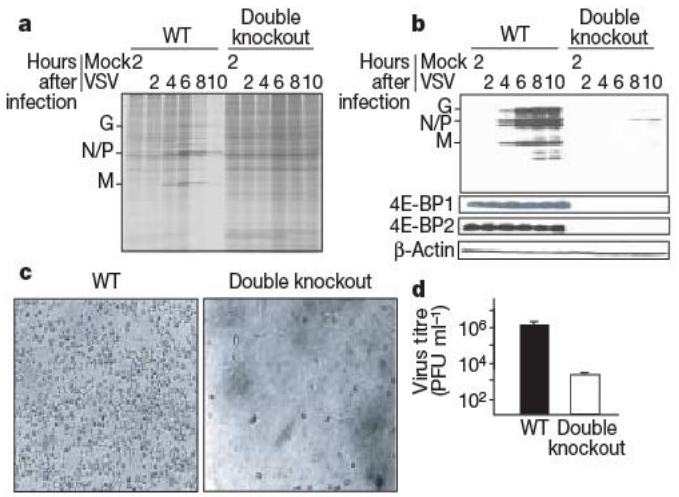


Figure 1 | Lack of 4E-BPs renders MEFs refractory to VSV replication. Wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs were mock-infected or infected with VSV at an MOI of 0.5 PFU per cell. **a**, MEFs were incubated with [^{35}S]methionine for 30 min at the indicated times after infection. Proteins were subjected to SDS–PAGE (15%). An autoradiogram of the dried gel is shown. Viral proteins are indicated on the left. G, glycoprotein; M, matrix protein; N/P, nucleocapsid protein/phosphoprotein. **b**, Western blotting analysis using antibodies against VSV proteins, 4E-BP1, 4E-BP2 and β-actin. **c**, **d**, Cytopathic effect (**c**) and virus yield (**d**) at 10 h after infection in wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs (mean ± s.d. of four experiments).

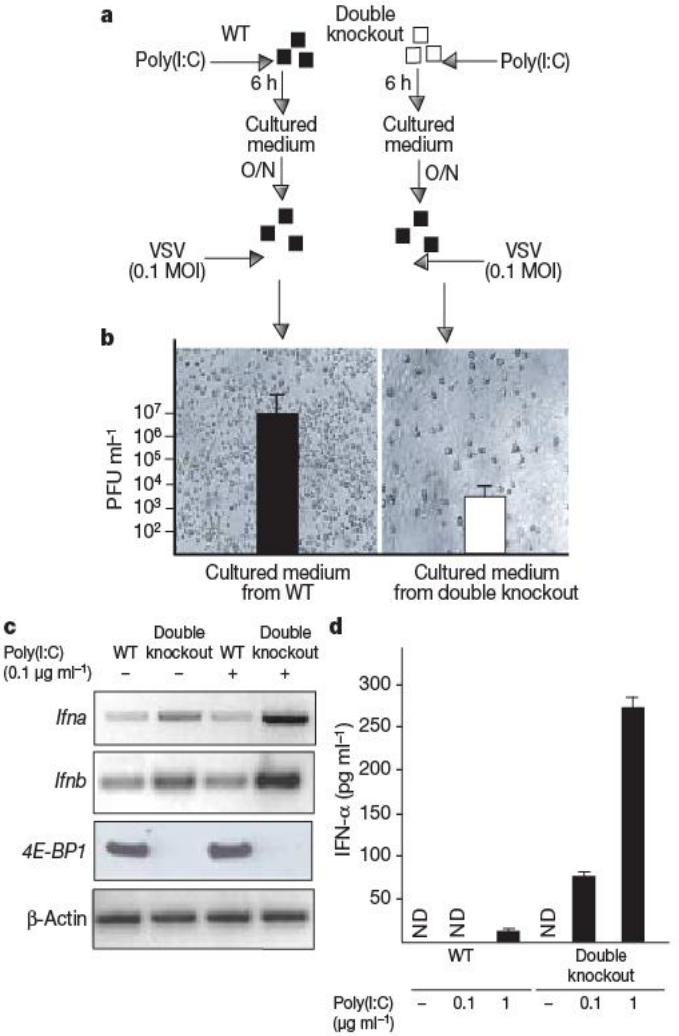


Figure 2 | Enhanced production of type-I IFN in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs. **a**, Diagram of experimental protocol. Wild-type (filled squares) and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ (open squares) MEFs were treated with poly(I:C) (0.1 μg ml^{-1}) for 6 h and medium was collected. Wild-type MEFs were incubated overnight (O/N) with the cultured medium from wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs and then infected with VSV. **b**, Cytopathic effect and virus titres at 24 h after infection. **c**, MEFs were treated with poly(I:C) for 6 h and the induction of *Ifna* and *Ifnb* mRNAs was determined by RT–PCR. **d**, MEFs were treated with poly(I:C) for 6 h and the production of IFN-β was determined by ELISA (mean ± s.d. of three experiments). ND, not detected.

VSV-infected *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice survived, in comparison to 20% of the wild-type mice (Fig. 3a). Furthermore, VSV-infected *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice, in contrast to wild-type mice, failed to exhibit severe respiratory distress. In a second experiment, mice were infected intranasally with VSV (10^5 PFU) and killed 5 days after infection. In lungs from *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice, virus load was reduced (~100-fold; Fig. 3b). The expression of both *Ifna* and *Ifnb* mRNAs, as assayed by RT–PCR, was significantly increased (~3-fold) already by 2 days after infection, as compared to wild-type mice (Fig. 3c). In addition, the serum of VSV-infected *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice contained increased IFN-α levels, as compared to the serum of VSV-infected wild-type mice (Supplementary Fig. 6). Thus, mice lacking 4E-BP1 and 4E-BP2 are resistant to VSV infection and produce more type-I IFN, as compared to wild-type mice.

Plasmacytoid dendritic cells are the main producers of systemic type-I IFN in response to virus infection[18]. To determine whether plasmacytoid dendritic cells from *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice contribute to the virus-resistant phenotype, splenic plasmacytoid dendritic cells were incubated with VSV (MOI of 1 and 10 PFU per cell) for 6 h. Plasmacytoid dendritic cells from *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice generated significantly more (>7-fold) IFN-α, as compared to plasmacytoid dendritic cells from wild-type littermates (Fig. 3d). Similarly, plasmacytoid dendritic cells from *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice, which were co-cultured with synthetic CpG-oligodeoxynucleotides (CpG-ODN), produced more IFN-α (>4-fold) than plasmacytoid dendritic cells from wild-type littermates (Fig. 3e). Thus, the virus-resistant phenotype of *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice correlates with the ability of their plasmacytoid dendritic cells to produce increased amounts of IFN-α in response to virus infection.

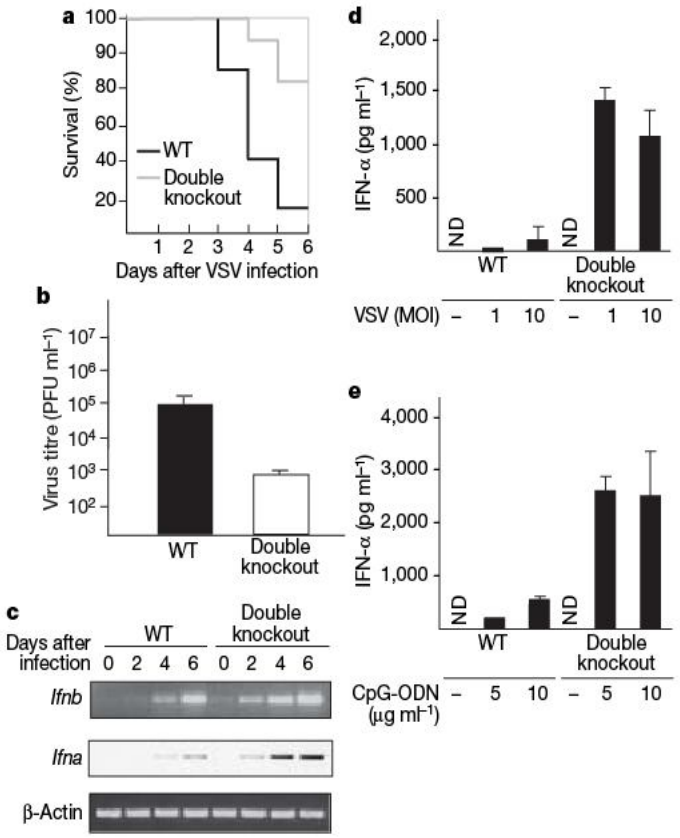


Figure 3 | *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice are resistant to VSV infection. **a**, Mice ($n = 10$) were intransally infected with VSV (5×10^7 PFU) and their survival was plotted as a Kaplan–Meier curve. **b**, Lungs from VSV-infected (10^5 PFU) mice ($n = 3$) were dissected 5 days after infection and virus yield was determined by plaque assay (mean ± s.d.). **c**, Expression of *Ifna* and *Ifnb* genes was determined by RT–PCR in lungs ($n = 3$). **d**, **e**, Splenic plasmacytoid dendritic cells were isolated and cultured for 6 h with VSV (**d**) or incubated overnight in the presence of CpG-ODN (**e**). Secreted IFN-α was measured by ELISA. ND, not detected.

Translational control of *Irf7* mRNA by 4E-BPs

To determine the molecular mechanism by which type-I IFN production is enhanced in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice, we used gene-expression microarrays. A number of genes in the IFN pathway were upregulated in uninfected *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ double knockout MEFs as compared to wild-type MEFs (Supplementary Fig. 7A; for a complete list of genes see Supplementary Fig. 7C). Furthermore, a number of genes involved in inflammation and the immune response were also upregulated (Supplementary Fig. 7A).

Because 4E-BPs are translational inhibitors, they are predicted to repress the translation of a subset of mRNAs that are critical for type-I IFN production. To identify these mRNAs, polysomal RNA from wild-type and double knockout MEFs was analysed by gene-expression microarrays. We identified mRNAs with low or no induction at the mRNA level (<1.5-fold), but with a robust induction of translation (>4-fold) (Supplementary Fig. 7B). Notably, *Irf7* mRNA, which encodes the master regulator of the type-I IFN response[19], was the highest ranked gene in this analysis as its expression was increased ~12-fold in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs as compared with wild-type MEFs (Supplementary Fig. 7B).

To validate these results, we studied the recruitment of ribosomes to *Irf7* mRNA using an RT–PCR assay that tracked the polysomal distribution of mRNAs in extracts from wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs along a sucrose density gradient (Fig. 4a). *Irf7* mRNA was mainly associated with light polysomes in wild-type MEFs (Fig. 4b, left panel), consistent with its inefficient translation initiation. β-Actin mRNA, by contrast, was distributed mainly in heavy polysomes, as expected for an mRNA that is translated efficiently (Fig. 4c). In agreement with the derepression of *Irf7* mRNA translation in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs, the distribution of *Irf7*

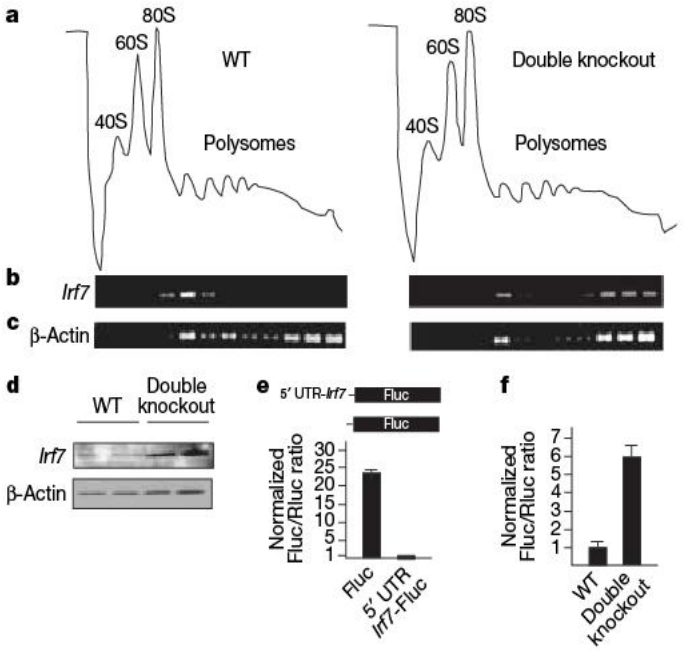
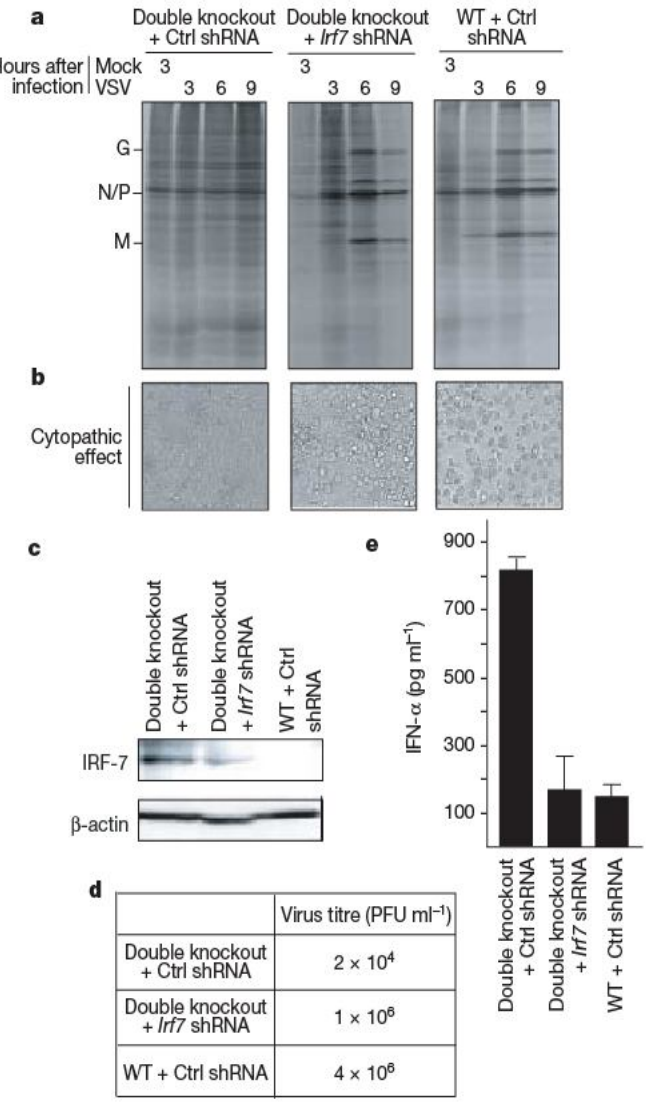


Figure 4 | 4E-BPs inhibit translation of *Irf7* mRNA. **a**, Polysome profiles of wild-type (left) and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ (right) MEFs. **b**, **c**, RT–PCR of *Irf7* (**b**) and β-actin (**c**) mRNAs. **d**, Western blot of IRF-7 and β-actin. **e**, Translation of 5′ UTR-*Irf7*-Fluc mRNA (Fluc, top) relative to Fluc mRNA. A *Renilla* luciferase (Rluc) reporter vector was co-transfected with both reporters as a transfection control. Fluc was normalized against Rluc. Values for the Fluc reporter were ~7×10^4 RLU (relative light units) and ~3×10^3 RLU for the 5′ UTR-*Irf7*-Fluc reporter. Rluc values were ~1×10^6 RLU. **f**, Ratio of expression of 5′ UTR-*Irf7*-Fluc/Rluc. The 5′ UTR-*Irf7*-Fluc and Rluc reporters were co-transfected. Fluc activity was normalized against Rluc activity. The Fluc value for wild-type MEFs was set as 1. For wild-type MEFs, Fluc ranged between 1×10^3 and 6×10^3 RLU and Rluc between 1.2×10^6 and 3.4×10^6. For *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs, Fluc ranged between 3.5×10^3 and 2.4×10^4 RLU and Rluc between 9×10^5 and 1.5×10^6 RLU.

mRNA was significantly shifted to heavier gradient fractions (Fig. 4b, right panel). Accordingly, in the absence of 4E-BP1 and 4E-BP2, IRF-7 protein amounts were increased (>4-fold), as determined by western blotting (Fig. 4d). These data support a role for the 4E-BPs in the repression of translation of *Irf7* mRNA.

Changes in the 4E-BPs/eIF4E ratio do not alter general translation, but rather affect the translation of a subset of mRNAs that harbour a structured 5′ untranslated region (5′ UTR)[7,20]. Notably, the *Irf7* mRNA has a highly structured 5′ UTR that is evolutionarily conserved (data not shown). To examine directly the role of the 5′ UTR in the regulation of *Irf7* mRNA translation, we generated a plasmid vector in which the SV40 promoter drives the expression of the 5′ UTR of *Irf7* mRNA fused to a firefly luciferase (Fluc) reporter (5′ UTR-*Irf7*-Fluc; Fig. 4e). As expected, because of the 5′ UTR secondary structure, the expression of 5′ UTR-*Irf7*-Fluc in wild-type MEFs was reduced relative to the control (lacking the 5′ UTR) Fluc reporter. A *Renilla* luciferase (Rluc) reporter plasmid was used as a transfection control (Fig. 4e). Next, wild-type and double knockout MEFs were co-transfected with 5′ UTR-*Irf7*-Fluc and an Rluc reporter plasmid. Consistent with the repression of *Irf7* mRNA translation by 4E-BPs, the normalized Fluc/Rluc ratio was significantly increased (~6-fold) in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs as compared with wild-type MEFs (Fig. 4f). Therefore, we conclude that the *Irf7* mRNA 5′ UTR has a critical role in its translational repression by 4E-BPs.

To determine whether the virus-resistant phenotype and the enhanced type-I IFN production in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs are due to increased IRF-7 expression, we reduced the IRF-7 level in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs using a short hairpin (sh)RNA against *Irf7* mRNA. shRNA against *Irf7* mRNA, but not a control shRNA, restored the sensitivity of these cells to VSV infection and blocked type-I IFN production (Fig. 5). These data provide genetic evidence that the enhanced type-I IFN response in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs is caused by upregulation of IRF-7 expression.



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	Virus titre (PFU ml^{-1})
Double knockout + Ctrl shRNA	2×10^4
Double knockout + *Irf7* shRNA	1×10^6
WT + Ctrl shRNA	4×10^6

Figure 5 | Reduction of IRF-7 in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs renders the cells susceptible to VSV infection and blocks type-I IFN production. **a**, *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs were first transfected with a control (Ctrl) shRNA or an shRNA against *Irf7* and then infected with VSV (MOI of 1 PFU per cell) and incubated with [^{35}S] methionine. Proteins were analysed by SDS–PAGE (15%). Viral proteins are indicated on the left. **b**, Cytopathic effect was visualized at 9 h after infection. **c**, Western blot analysis using antibodies against IRF-7 and β-actin. **d**, Virus yield was determined at 9 h after infection. **e**, IFN-α was determined 9 h after infection by ELISA (mean ± s.d. of three experiments).

Discussion

Virus infection results in the induction of type-I IFN, which confers an antiviral state on the host cell. New expression of IFN-related genes is required for the activation of the innate immune response[21,22]. The balance between activators and repressors of type-I IFN response is critical for this process. Until now, studies described only positive regulators of the innate immune response. For instance, *Stat1* and *Stat2* knockout mice[23–25] and RNase L (*Rnasel*) knockout mice[26] exhibit increased sensitivity to virus infections because of impaired IFN response. Knockout mice for two virus cytoplasmic sensors, retinoic-acid-inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA5), display a similar phenotype[27,28]. Our results provide new insight into the molecular mechanism of the innate immune response by providing strong evidence for a repressive translational mechanism that impedes type-I IFN production. We show that MEFs lacking 4E-BPs are largely refractory to infection by a variety of viruses (Fig. 1 and Supplementary Fig. 3). The virus-resistant phenotype is due to an increased type-I IFN production, as determined by inhibition of VSV replication, as well as RT–PCR, microarray and ELISA analyses (Fig. 2 and Supplementary Figs 4 and 5). In *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ double knockout mice VSV replication was suppressed. The VSV-resistant phenotype correlates with enhanced production of IFN-α in plasmacytoid dendritic cells (Fig. 3). These data indicate a causative role of the enhanced expression of type-I IFN in plasmacytoid dendritic cells in the resistance of *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice to VSV infection.

It is thought that plasmacytoid dendritic cells produce large amounts of type-I IFN as a consequence of a high constitutive expression of IRF-7 (ref. 18). Consistent with the control of *Irf7* mRNA translation by 4E-BPs, we demonstrated that the level of 4E-BP1 and 4E-BP2 in plasmacytoid dendritic cells is significantly lower as compared to MEFs (Supplementary Fig. 8). Thus, the low levels of 4E-BPs could explain the constitutive expression of IRF-7 in plasmacytoid dendritic cells.

Other lines of evidence support the idea that downstream targets of mTOR such as 4E-BPs, S6K1/2 or PRAS40 have an important role in repression of IFN production. First, *4E-BP1*$^{-/-}$ MEFs primed with mouse IFN exhibit enhanced expression of IFN-related genes[29]. Second, activation of toll-like receptor 3 (TLR3), which is a major mediator of the cellular response to virus infection through dsRNA, activates phosphatidylinositol-3-OH kinase (PI(3)K), an upstream regulator of 4E-BPs[30]. Interestingly, PI(3)K-γ and PI(3)K-δ knockout mice exhibit a defect in innate immunity[31]. In addition, pharmacological and genetic inhibition of PI(3)K blocks the induction of IFN-stimulated genes by dsRNA[32]. Third, similar to the phenotype of the *4E-BP1*$^{-/-}$ cells, the expression of type-I IFN genes was enhanced in MEFs lacking the mTOR negative regulator, tuberous sclerosis complex 2, when primed with mouse IFN[29].

How do 4E-BPs regulate type-I IFN production? Our model (Supplementary Fig. 9) is based on the translational control of *Irf7* mRNA by the 4E-BPs. *Irf7* mRNA translation is derepressed in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs (Fig. 4) owing to increased amounts of the eIF4F complex. Because of its highly structured 5′ UTR, the translation of *Irf7* mRNA would be extremely sensitive to changes in the

amounts of eIF4F. In agreement with this model, translation of a reporter mRNA harbouring the 5′ UTR of *Irf7* mRNA is enhanced in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs. An increase in IRF-7 expression triggers type-I IFN production, which subsequently evokes a transcriptionally dependent positive feedback regulation of IRF-7 signalling[33,34]. Consistent with this, we showed that the expression of IFN-α and IFN-β is enhanced (Fig. 2 and Supplementary Figs 4 and 5) and IRF-7 levels are upregulated in uninfected *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs (Fig. 4 and Supplementary Fig. 7B). Enhanced type-I IFN production in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs seems to be highly dependent on IRF-7, as decreasing IRF-7 levels by shRNA rendered cells susceptible to virus infection and blocked type-I IFN production (Fig. 5). Accordingly, *Irf7*$^{-/-}$ mice exhibit increased susceptibility to virus infection both *in vivo* and *ex vivo* as well as impaired induction of type-I IFN[19]; that is, a phenotype opposite to that of the *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice. The activation of the IRF-7-mediated type-I IFN response induces the expression of RIG-I and MDA5, which triggers the induction of NF-κB, IRF-3 and IRF-7 that co-operate in the production of the antiviral type-I IFN response. As expected from this model, we found enhanced expression of RIG-I and MDA5 proteins in MEFs lacking 4E-BPs (Supplementary Fig. 10).

Our data provide biochemical, genetic and biological evidence that 4E-BPs constitute a critical step in the activation of the innate immune response. These findings raise the intriguing possibility that regulators of translation might serve as therapeutic targets to boost the innate immune response against virus infection.

METHODS SUMMARY

Mice, cell culture and viruses. *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ double knockout mice have been described previously[15]. MEFs derived from wild-type and *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ mice were immortalized by sequential passaging[35]. Splenic plasmacytoid dendritic cells were isolated using anti-mPDCA1 magnetic beads. *Ex vivo* virus infection and metabolic labelling were performed as described[36]. Virus titres were determined by a plaque assay[37,38]. *In vivo* virus experiments were performed as described[38].

Polysome profiling. Polysomes were prepared and analysed as described[39]. *Irf7* and β-actin mRNAs were amplified by RT–PCR reactions, which were optimized to measure the exponential phase on the amplification curve.

Microarray analysis. Total or polysomal RNA was isolated from MEFs using Trizol and hybridized to an Affymetrix Mouse430_2 chip. To identify genes upregulated in *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs, total RNA samples were analysed using normalization that reduced the range for the fold change (>1.5-fold used as threshold). Translationally upregulated genes were identified by selecting genes whose regulation in total RNA samples was low (<1.5-fold), but their abundance on polysomes was >4-fold.

ELISA. MEFs were transfected with poly(I:C) using the FuGENE 6 transfection reagent according to the manufacturer's protocol (Roche). Murine IFN-α and IFN-β production was detected by ELISA according to the manufacturer's procedure (PBL Biomedical Laboratories).

Plasmid construction, transfection and luciferase assay. The 5′ UTR of mouse *Irf7* mRNA was amplified and cloned into the pGL3 firefly luciferase (Fluc) reporter vector (Promega). MEFs were co-transfected with 5′ UTR-*Irf7*-Fluc and *Renilla* luciferase (Rluc; Promega) as described[36]. Cell extracts were prepared in passive lysis buffer and assayed for Rluc and Fluc activity using a dual-luciferase reporter assay system (Promega). Fluc activity was normalized against Rluc activity, which was used as a transfection control.

Rescue experiments. pBABE-4E-BP1, pBABE-4E-BP2 and empty vector were transfected into phoenix-293-T packaging cells and virus-containing medium was used to infect *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs. *4E-BP1*$^{-/-}$ *4E-BP2*$^{-/-}$ MEFs were transfected with PLKO.1-puro-Ctrl-shRNA or PLKO.1-puro-*Irf7*-shRNA as described[36]. Transfected MEFs were selected with puromycin for 1 week.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 28 November 2007; accepted 25 January 2008.
Published online 13 February 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank M. Karin, M. Gale, R. Lin, W. Sossin, L. W. Ler and A. Rosenfeld for comments on the paper, and N. Taheri, A. Sylvestre and C. Lister for assistance. RIG-I and MDA5 antibodies were provided by H. Kato. This work was supported by a grant from the National Cancer Institute of Canada to N.S. and J.C.B. N.S. is a Howard Hughes Medical Institute (HHMI) International scholar. R.C. is supported by a Cole Foundation post-doctoral fellowship and R.J.O.D. is supported by a Terry Fox Foundation studentship.

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LETTERS

The presence of methane in the atmosphere of an extrasolar planet

Mark R. Swain[1]*, Gautam Vasisht[1]* & Giovanna Tinetti[2]*

Molecules present in the atmospheres of extrasolar planets are expected to influence strongly the balance of atmospheric radiation, to trace dynamical and chemical processes, and to indicate the presence of disequilibrium effects. As molecules have the potential to reveal atmospheric conditions and chemistry, searching for them is a high priority. The rotational–vibrational transition bands of water, carbon monoxide and methane are anticipated to be the primary sources of non-continuum opacity in hot-Jupiter planets[1–3]. As these bands can overlap in wavelength, and the corresponding signatures from them are weak, decisive identification requires precision infrared spectroscopy. Here we report a near-infrared transmission spectrum of the planet HD 189733b that shows the presence of methane. Additionally, a resolved water vapour band at 1.9 μm confirms the recent claim[4] of water in this object. On thermochemical grounds, carbon monoxide is expected to be abundant in the upper atmosphere of hot-Jupiter planets, but is not identifiable here; therefore the detection of methane rather than carbon monoxide in such a hot planet[5,6] could signal the presence of a horizontal chemical gradient away from the permanent dayside, or it may imply an ill-understood photochemical mechanism that leads to an enhancement of methane.

To date, molecular signatures have not been resolved in the emission spectra of hot-Jupiter extrasolar planets[7–10] (exoplanets). Transmission spectroscopy during the primary eclipse (when the planet occults a portion of the stellar disk, and a fraction of light from the star is seen after traversal through the atmosphere around the planet's limb) has the advantage of being insensitive to temperature structure in the exoplanet's atmosphere[11]. Owing to the presence of strong molecular absorption bands, near-infrared spectroscopy from 1 to 2.5 μm is well suited for detection of the signatures of H_2O, CO and CH_4. For a hot-Jupiter atmosphere in purely thermochemical equilibrium, the dominant carbon-bearing molecule is expected to be CO at higher temperatures (temperature $T > 1{,}200$ K) and CH_4 at lower temperatures ($T < 800$ K). On the daysides of the short-period, tidally locked hot-Jupiters, the local carbon chemistry should be dominated by CO; disequilibrium effects may result in CO as the dominant carbon-carrying molecule, even on the terminators and nightsides of such planets[6]. Our detection of the onset of the CH_4 bandhead at 2.2 μm is the first clear spectral signature of a carbon-based molecule in an exoplanet atmosphere.

We observed the transiting exoplanet HD 189733b with the Hubble Space Telescope using the NICMOS (NIC-3) camera on 25 May 2007, over five contiguous spacecraft orbits. During these observations, NICMOS was configured to obtain a spectrophotometric time series between 1.4 and 2.5 μm. As the parent star, HD 189733, is extremely bright (K-band magnitude $K = 5.52$), we defocused the NICMOS camera to increase the saturation time of individual detector pixels. This provided additional benefits for precision photometry[12] by allowing some spatial averaging over intra-pixel and pixel-to-pixel quantum efficiency variations that could couple to rapid telescope pointing errors and slow pointing drifts to produce spurious intensity fluctuations in the measured time series. The defocus determines the spectral resolution, allowing us to extract 18 independent wavelength channels with sufficient signal-to-noise ratio. There are gaps in the measured time series because the star HD 189733 is not in the 'continuous viewing zone' of the spacecraft, and, because of scheduling constraints, the in-eclipse data are not symmetric about the epoch of inferior conjunction.

During primary eclipse, the apparent brightness of the star decreased by ~2.4% (Fig. 1). However, as the modulation amplitude due to nominal amounts of water vapour[13] is predicted to be small (<0.1%), high-dynamic-range (~10^4) spectra are required to detect and characterize any molecular features. Although the Hubble Space Telescope avoids the limitations imposed by the Earth's atmosphere, spectrophotometry with NICMOS or other on-board instruments[12,14] is subject to systematic errors that must be corrected

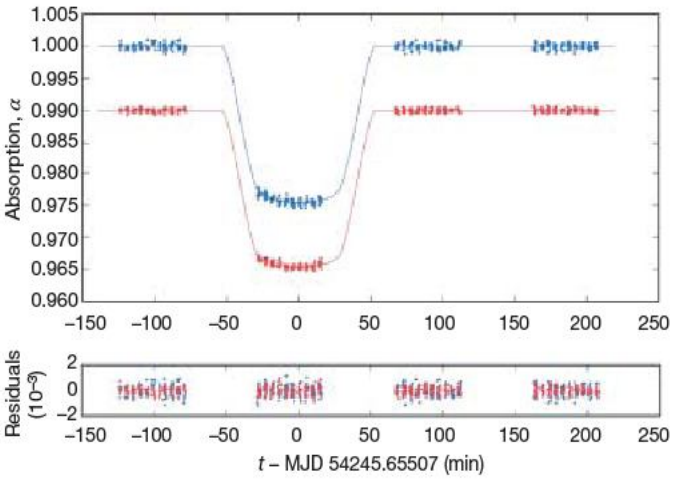

Figure 1 | Calibrated measurements showing the primary eclipse event. Top panel, the measured time series (where α is the percent absorption and t is time in units of minutes of modified Julian day, MJD), corrected for systematic errors, taken during four orbits of the Hubble Space Telescope. The primary eclipse occurs during the second orbit, and the curvature of the eclipse light curve is due to limb darkening of the star. The two time series shown are co-added spectral data approximately matching the H and K astronomical bands (blue, 1.6–1.8 μm; red, 2.0–2.4 μm). The gaps in the time series are because HD 189733 is not in the continuous viewing zone for the Hubble Space Telescope. For clarity, the red light curve has been offset. Bottom panel, residuals that correspond to the difference between the measured light curve and the model.

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because the errors are similar in size to the expected molecular signatures. In our data, the first orbit had strong systematic offsets (due to spacecraft settling) and was excluded from our analysis.

In order to arrive at the transmission spectrum, it is important to establish a proper baseline for the remaining out-of-eclipse data. The systematic errors in the raw light curves are dominated by two types, correlations in time and in wavelength. In order to remove temporal correlations, we assumed that the observed flux in each wavelength channel for the out-of-eclipse orbits could be modelled by perturbations that were linear in five state variables, and by a term that was up to parabolic in spacecraft orbital phase. The state variables capture the optical state of the camera and are the centroids of channels, a variable defocus due to 'breathing' of the telescope focus, rotation of the spectrum with respect to the detector, and temperature. A regression to the observed light curves provided the coefficients of the model. When decorrelated on the basis of the model, the time series showed no further temporal correlations. Some remaining excess noise was strongly correlated in wavelength; an estimator for this noise was constructed as a weighted average of all channel time series data (collapsed in the wavelength axis) and was then subtracted from individual channels. The resulting channel time series are near the theoretical noise limit. The robustness of the fits was verified by removing sections of the data from the fit and ensuring that the combined residuals remain well behaved.

The in-eclipse time series temporal effects were decorrelated by applying the model coefficients determined from the out-of-eclipse data to state variables and the spacecraft orbital phase at every time-step during that orbit. After accounting for the chromatic effects introduced in the eclipse light curve by limb darkening, a wavelength decorrelation was performed using the same method as was used for the out-of-eclipse orbits. The transit time series, now also corrected for instrument systematics, was averaged to construct a transmission spectrum. This spectrum includes astrophysical biases that have weak chromatic dependence, due to averaging over limb-darkened light curves and also due to the presence of cool starspots on the disk of the star. The effect of limb darkening, though not as dramatic as in optical data, is clearly seen as curvature in the eclipse light curves (Fig. 1). We co-added the spectral channels to construct synthetic light curves matching the H and K astronomical bands, for which there are published nonlinear, limb-darkening laws[15]. We then constructed a computer model of a limb-darkened star, and simulated the motion of the planet across the stellar disk using the system's keplerian parameters[14,16] to generate model light curves. A steepest descent scheme was used to fit generated curves to the measured data by leaving the planet effective radius (in that band) and epoch of transit as free parameters. The best-fit light curves in each band were used to estimate the limb-darkening biases; the mean correction is 2×10^{-4}, and the colour correction is 1×10^{-4} across the band. The presence of starspots on HD 189733 has been inferred[16] from measurements of long-term, out-of-eclipse variability, and from the structure of transit light curves at optical wavelengths[14]. Unocculted starspots introduce a positive chromatic bias in the inferred absorption depth, as they are cooler than the stellar disk; in the infrared, the chromaticity of this effect is small and monotonic, and thus has little effect on the shape of the observed modulation. We derived a correction with a starspot coverage of 4% using model spectra[17] and assuming that the starspots were on average 1,000 K cooler than the 5,000 K stellar photosphere. A more detailed description of the data reduction method is available (Supplementary Information).

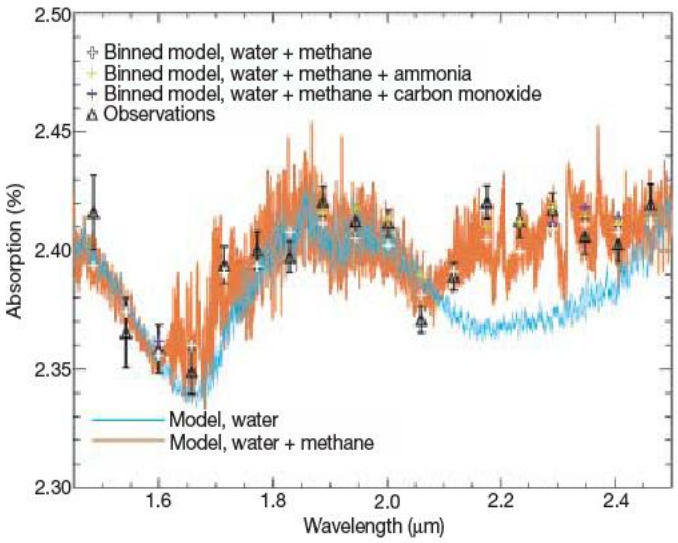

Figure 2 | A comparison of observations with simulated water and methane absorption. The measured spectrum (black triangles), and two theoretical spectra of the predominantly H_2 atmosphere, showing the effects of small amounts of water (blue) and methane in combination with water (orange). The measured spectrum contains significant differences at 1.7–1.8 μm and at 2.15–2.4 μm from what is expected due to water vapour alone. We interpret these departures as additional absorption features due to the presence of one or more other species in addition to water. When considering only water and methane, the theoretical spectrum best fitting the data was determined by binning the model (shown as white crosses) to the spectral resolution of the observations. Different model predictions based on changing abundances and molecules were compared to the observations using the reduced χ^2; the best fitting model has a water abundance of 5×10^{-4} and a methane abundance of 5×10^{-5}. The model spectrum can be improved slightly with the addition of small ($\sim 1 \times 10^{-5}$) amounts of either ammonia or carbon monoxide (shown in green and purple crosses, respectively). Error bars show $\pm 1\sigma$; the error includes the uncertainty in the correction of systematic effects (see Supplementary Information). Note that determining the zero point for the spectrum depends on the diameter assumed for the planet and assumptions in the starspot correction. Thus, the shape of the spectrum is robust; there is an uncertainty of $\pm 2 \times 10^{-4}$ in the absolute level.

We show the corrected spectrum as relative absorption depth in Fig. 2. The signature of the H_2O absorption band centred around 1.9 μm is immediately obvious. The steep increase in absorption at the short-wavelength edge is also most probably due to an adjacent water band centred at a wavelength less than 1.5 μm. Thus, the new spectrum allows an unambiguous identification of water vapour in the atmosphere of HD 189733b, confirming its earlier inference from broadband photometry[4]. As a steep change in absorption occurs at 2.2 μm, the observations decisively show that methane is present in addition to water. To explore the abundance of H_2O and CH_4 and possible contributions of CO and NH_3, simulated transmission spectra were generated using a recent version of a planetary spectral model[13]. The model covers a range of pressure from ~10 to $\sim 10^{-10}$ bar and includes transitions[18] for H_2–H_2 (the most common molecular species). The temperature and density at each atmospheric level are determined by the pressure–temperature profile, and the absorption contribution from each molecule is computed on the basis of its mixing ratio. The H_2O absorption coefficients incorporate a new, high-accuracy line list[19], and the CO absorption coefficients were estimated with the HITEMP data base (L. S. Rothman *et al.*, personal communication). The CH_4 absorption coefficients were evaluated using a combination of line lists[20,21]. For all the molecules, the opacities are calculated for the selected spectral band at the different temperatures of the atmospheric layers (from 500 K to 2,000 K) and in some cases[18,21] interpolated for intermediate values of the available temperatures. We used the 'evening terminator' pressure–temperature profile[22]; significantly different temperature profiles produce results that do not match the observed water vapour absorption features. The theoretical spectra were binned to the same spectral resolution as the measurements, and the results of different compositions were compared with the observations using the reduced χ^2 value. Combinations of H_2O and CO, as well as H_2O and NH_3, failed to match the observed spectrum. The model best fitting the observations has a mixing ratio of $\sim 5 \times 10^{-4}$ for H_2O, and

5×10^{-5} for CH_4 (see Fig. 2); the addition of NH_3 with a mixing ratio of 1×10^{-5} improves the fit slightly. The agreement between our H_2O mixing ratio value and previous results[4] is significant because a wide range of wavelengths, covering three major H_2O absorption bands, can be modelled self-consistently; this implies that the estimated H_2O mixing ratio is robust. The pressure at which the atmosphere becomes optically thick ranges from a few millibars, when the absorption is strong, to ~0.2 bar, when the absorption is weaker (for example, ~1.7 μm). We have modelled the effect of aerosols and determined that our spectrum is haze-free, as their contribution would depress the spectral signature of both H_2O and CH_4 relative to the measured absorption depth. If aerosols are present, as is suggested by recent measurements[23], they must be in the form of small particles and their effects confined to wavelengths shorter than 1.5 μm (ref. 24).

Although we can unambiguously determine that CH_4 is present, the CH_4 abundance estimate is dependent on uncertainties in the high-temperature transitions. Additionally, the presence of CH_4 masks the effect of CO in the absorption spectrum. Although the best fit is obtained using only H_2O and CH_4, CO can be included up to the abundance of H_2O with a modest increase in χ^2. Thus, we cannot strongly constrain abundance of CO or the elemental C/O ratio. The relatively high concentration of CH_4 could be due in part to photochemistry. Photolysis of CO and CH_4 is probably not significant in the upper atmosphere (pressure $P < 10^{-4}$ bar) of HD 189733b because of the strength of C–O and C–H bonds and the deficit of ultraviolet flux from its cool parent star (spectral type K2V). However, the photolysis of the weaker bonds of sulphur-, nitrogen- and oxygen-bearing compounds and the accompanying availability of fast-reacting free radicals could have a significant effect on the relative abundance[25] of CH_4 compared to CO.

Received 14 September 2007; accepted 6 February 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank D. Deming for contributions to the original proposal and for suggestions for clarifying the material presented in the manuscript. We thank T. Wiklind, N. Pirzkal and other members of the Space Telescope Science Institute staff for assistance in planning the observations and for advising how the observations could be optimized. We also thank K. Jahnke for suggesting the long-wavelength NICMOS grism, F. Pont for discussions concerning the treatment of the data, M.-C. Liang for discussions on photolysis, and J. Tennyson and R. Barber for suggestions on the methane data lists. G.T. was supported by the UK Sciences & Technology Facilities Council and the European Space Agency. The research described in this paper was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA.

Author Contributions M.R.S. was the PI of the project and led the overall direction of the research. G.V. led the data analysis and G.T. led the modelling. All authors contributed equally to the writing of the paper.

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LETTERS

Difference in direct charge-parity violation between charged and neutral *B* meson decays

The Belle Collaboration*

Equal amounts of matter and antimatter are predicted to have been produced in the Big Bang, but our observable Universe is clearly matter-dominated. One of the prerequisites[1] for understanding this elimination of antimatter is the nonconservation of charge-parity (CP) symmetry. So far, two types of CP violation have been observed in the neutral K meson (K^0) and B meson (B^0) systems: CP violation involving the mixing[2] between K^0 and its antiparticle $\bar{K}^0$ (and likewise[3,4] for B^0 and $\bar{B}^0$), and direct CP violation in the decay of each meson[5–8]. The observed effects for both types of CP violation are substantially larger for the B^0 meson system. However, they are still consistent with the standard model of particle physics, which has a unique source[9] of CP violation that is known to be too small[10] to account for the matter-dominated Universe. Here we report that the direct CP violation in charged $B^{\pm}\to K^{\pm}\pi^0$ decay is different from that in the neutral B^0 counterpart. The direct CP-violating decay rate asymmetry, $\mathcal{A}_{K^{\pm}\pi^0}$ (that is, the difference between the number of observed $B^-\to K^-\pi^0$ event versus $B^+\to K^+\pi^0$ events, normalized to the sum of these events) is measured to be about +7%, with an uncertainty that is reduced by a factor of 1.7 from a previous measurement[7]. However, the asymmetry $\mathcal{A}_{K^{\pm}\pi^{\mp}}$ for $\bar{B}^0\to K^-\pi^+$ versus $B^0\to K^+\pi^-$ is at the −10% level[7,8]. Although it is susceptible to strong interaction effects that need further clarification, this large deviation in direct CP violation between charged and neutral B meson decays could be an indication of new sources of CP violation—which would help to explain the dominance of matter in the Universe.

Existing measurements of CP asymmetries in K and B meson decays can be explained using a single source of CP violation from the mechanism of the Kobayashi–Maskawa model. Proposed[9] in 1973, this mechanism anticipated the third family of quarks before they were discovered. Together with a quantum field theory that describes the electromagnetic, weak and strong interactions, it is a key part of the standard model of particle physics. The present Kobayashi–Maskawa source of CP violation, however, is itself too small (see ref. 10 for example) to account for the dominance of matter in the Universe. A search for other sources of CP violation, in the neutrino sector or in new physics beyond the standard model, is needed.

The decay $B\to K\pi$ proceeds through two major processes, illustrated in Fig. 1a and b. Figure 1a is called the colour-allowed tree diagram, and the Kobayashi–Maskawa source of CP violation enters via the so-called V_{ub} (where ub represents the transition between u and b quarks) matrix element that governs the $\bar{b}u\bar{W}$ interaction vertex. On the other hand, while all charge 2/3 quarks contribute to the quantum 'loop', it is the virtual top quark that dominates the amplitude of the process shown in Fig. 1b, which is usually called the (strong) penguin diagram. The controlling matrix element product $V_{tb}V_{ts}^*$ (where tb and ts represent the transitions between t and b quarks and t and s quarks) is insensitive to the Kobayashi–Maskawa source of CP violation. CP violation may arise from the interference between these two amplitudes, similar to two waves interfering with each other to produce a combined wave. However, this still depends on the detailed dynamics of each process. It is a theoretical challenge to describe how the quark level decay evolves into the observed mesons. One of the advantages of studying a direct CP-violating asymmetry, which is a ratio of decay rates, is that many of the experimental systematic uncertainties cancel. Consequently, CP-violating asymmetries provide information about the dynamics of B meson decay, test different theoretical approaches, and probe new physics beyond the standard model.

Compared to the dominant $b\to c$ decay amplitudes, the amplitude of Fig. 1a is suppressed by the smallness of $|V_{ub}/V_{cb}|$, while Fig. 1b is suppressed by the quantum loop amplitude. However, the two amplitudes are of similar magnitude, allowing for large interference (and hence appreciable CP violation) to occur. The price to pay is the small branching fractions or decay rates to be measured. For instance, out of a million neutral B^0 mesons, only about 20 will decay into $K^+\pi^-$, while for B^+ mesons, only about 13 in a million will decay to $K^+\pi^0$. Therefore, to search for CP violation, we must produce many B mesons and detect them with high efficiency. The Belle detector at the KEKB[11] asymmetric-energy (3.5 on 8.0 GeV) e^+e^- collider, operating on the $\Upsilon(4S)$ resonance (which decays exclusively to a $B\bar{B}$ meson pair) energy, was designed for such a purpose. The KEKB accelerator is currently the brightest collider in the world, in which the record instantaneous luminosity is equivalent to bombarding a 1 cm^2 area with 1.7×10^{34} particles per second. A detailed description of the Belle detector (see Supplementary Information 1) can be found elsewhere[12]. Here we report our measurements of CP-violating asymmetries for the $B\to K^{\pm}\pi^{\mp}$, $K^{\pm}\pi^0$ and $\pi^{\pm}\pi^0$ modes, using 535 million $B\bar{B}$ meson pairs collected with the Belle detector.

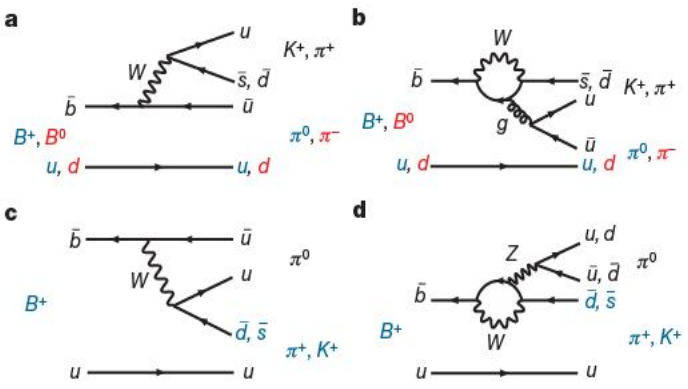

Figure 1 | Feynman diagrams for $B\to K\pi, \pi\pi$. The $B^+(B^0)$ meson consists of a $\bar{b}$ quark and a $u(d)$ quark, while its antiparticle, $B^-(\bar{B}^0)$ is made of a b quark and a $\bar{u}(\bar{d})$ quark. Contributions from diagrams **a** and **b** are expected to be dominant over those from **c** and **d**.

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Candidate B mesons are reconstructed by pairing a charged kaon or pion with another pion of opposite charge or with a neutral pion. Two variables are used to identify B candidates: the beam-energy constrained mass, $M_{\rm bc}=\sqrt{E^2_{\rm beam}-P^2_B}$, and the energy difference, $\Delta E=E_B-E_{\rm beam}$, where $E_{\rm beam}$ is the $e^{\pm}$ beam energy and E_B and P_B are the reconstructed energy and momentum of the B candidate in the e^+e^- centre-of-mass frame. Real B meson events give $M_{\rm bc}\cong 5.28\ {\rm GeV}/c^2$ and $\Delta E\cong 0\ {\rm GeV}$ while background events are distributed differently. Using a continuum suppression[7] method to reduce background arising from $e^+e^-\rightarrow q\bar{q}$ (where $q=u$, d, s and c quarks), the number of signal B mesons and CP asymmetry are extracted by performing an unbinned maximum-likelihood fit to the $M_{\rm bc}-\Delta E$ distribution with expected signal and background shapes (as illustrated in Fig. 2 for $M_{\rm bc}$).

Figure 2a and b shows the $M_{\rm bc}$ projections for the $B\rightarrow K^{\pm}\pi^{\mp}$ candidates. In 535 million $B\bar{B}$ pairs, we observe $2{,}241\pm57$ $K^+\pi^-$ and $1{,}856\pm52$ $K^-\pi^+$ signal events. The CP-violating asymmetry in $B\rightarrow K^{\pm}\pi^{\mp}$ is measured to be:

$$\mathcal{A}_{K^{\pm}\pi^{\mp}}\equiv\frac{N(\bar{B}^0\rightarrow K^-\pi^+)-N(B^0-K^+\pi^-)}{N(\bar{B}^0\rightarrow K^-\pi^+)+N(B^0\rightarrow K^+\pi^-)}=-0.094\pm0.018\pm0.008, \quad (1)$$

where $N(\bar{B}^0\rightarrow K^-\pi^+)$ is the yield obtained for the $\bar{B}^0\rightarrow K^-\pi^+$ decay and $N(B^0\rightarrow K^+\pi^-)$ denotes the yield of the antiparticle mode. The first error in the measurement is statistical, while the second is the systematic error from fitting and bias due to detector response (as it is made from matter, not antimatter). The latter is investigated using a large sample of tagged $D\rightarrow K^{\pm}\pi^{\mp}$ decays (with K and π momenta in the same kinematic region as B decays), where no CP-violating asymmetry is expected. No obvious bias is observed. Furthermore, the obtained background asymmetry of -0.005 ± 0.003 from the fit to the B candidates is consistent with zero, implying that detector bias is small. Equation (1) corresponds to a significance of 4.8σ, or a probability for no asymmetry of less than 1.8×10^{-6}. The result is consistent with the measurements by the BaBar[8,13] and CDF[14] collaborations, as well as with our previous measurement[7], which used 275 million $B\bar{B}$ pairs. The observed sign and strength of $\mathcal{A}_{K^{\pm}\pi^{\mp}}$ were anticipated by the perturbative QCD factorization approach[15], while the QCD factorization approach[16] predicted the opposite sign.

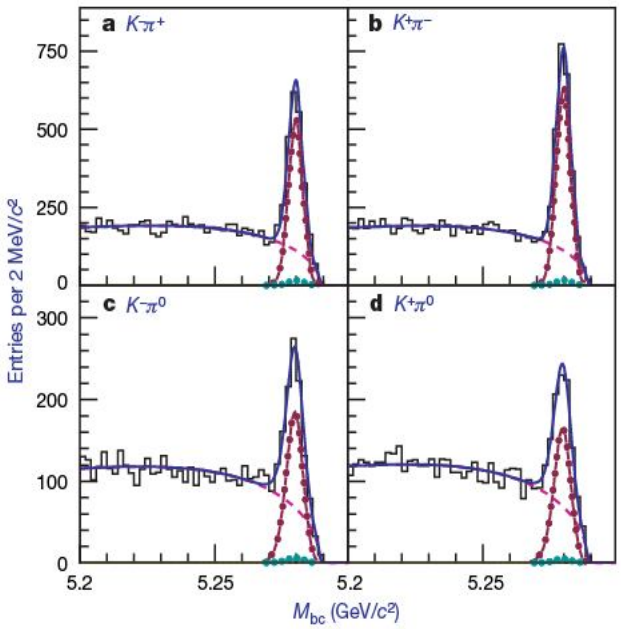

Figure 2 | $M_{\rm bc}$ projections for $K^-\pi^+$ (a), $K^+\pi^-$ (b), $K^-\pi^0$ (c) and $K^+\pi^0$ (d). Histograms are data, solid blue lines are the fit projections, point-dashed lines are the signal components, dashed lines are the continuum background, and grey dotted lines are the $\pi^{\pm}\pi$ signals that are misidentified as $K^{\pm}\pi$. The $M_{\rm bc}$ projections are made by requiring $|\Delta E|<0.06$ GeV for $K^{\pm}\pi^{\mp}$ and $-0.14<\Delta E<0.06$ GeV for $K^{\pm}\pi^0$.

For the decay final states with a π^0, a similar procedure gives $1{,}600^{+57}_{-55}$ $K^{\pm}\pi^0$ and 735^{+44}_{-43} $\pi^{\pm}\pi^0$ signal events, with the associated asymmetries of:

$$\mathcal{A}_{K^{\pm}\pi^0}=+0.07\pm0.03\pm0.01, \quad (2)$$

$$\mathcal{A}_{\pi^{\pm}\pi^0}=+0.07\pm0.06\pm0.01. \quad (3)$$

In the $M_{\rm bc}$ projection plots of Fig. 2c and d, slightly more B^- signal events compared with B^+ events are apparent, in contrast to the behaviour in Fig. 2a compared to Fig. 2b. Equations (2) and (3) are also in agreement with previous measurements[7,17], but more precise. With our new measurements of $\mathcal{A}_{K^{\pm}\pi^{\mp}}$ and $\mathcal{A}_{K^{\pm}\pi^0}$, the difference between direct CP violation in charged and neutral B meson decays into $K\pi$ is:

$$\Delta\mathcal{A}\equiv\mathcal{A}_{K^{\pm}\pi^0}-\mathcal{A}_{K^{\pm}\pi^{\mp}}=+0.164\pm0.037, \quad (4)$$

which is now established at the 4.4σ level; the probability for no difference is less than 9.3×10^{-6}. We note that in our previous measurement[7], based on 275 million $B\bar{B}$ pairs, the significance of the difference was only 2.4σ (1.9×10^{-2} null probability), a statistically marginal effect that could have disappeared by adding an equivalent amount of data (but did not in our case).

What is the interpretation of the difference between $\mathcal{A}_{K^{\pm}\pi^{\mp}}$ and $\mathcal{A}_{K^{\pm}\pi^0}$? For the decay $B^{\pm}\rightarrow\pi^{\pm}\pi^0$, the contribution from the penguin diagram of Fig. 1b vanishes by isospin symmetry. With Fig. 1a as the single dominant amplitude, the CP-violating asymmetry is expected to be very small. Given the current errors, our measurement of $\mathcal{A}_{\pi^{\pm}\pi^0}$ is consistent with this expectation. On the other hand, both Fig. 1a and b contribute to $B\rightarrow K^{\pm}\pi^{\mp}$ and $B^{\pm}\rightarrow K^{\pm}\pi^0$ and we would expect[15,16] $\mathcal{A}_{K^{\pm}\pi^{\mp}}$ and $\mathcal{A}_{K^{\pm}\pi^0}$ to be rather close to each other. However, we find not only a significant difference in magnitude but also a sign difference between the central values of equation (2) and equation (1). There are several theoretical conjectures that try to explain this $K\pi$ asymmetry puzzle: enhancement of the colour-suppressed tree amplitude[18,19] (Fig. 1c), electroweak penguin contributions[20] (Fig. 1d, which is Fig. 1b with the gluon g replaced by Z), or both[21]. If this effect were to be explained solely by enhancement of the colour-suppressed tree amplitude (which is also proportional to V_{ub}), its amplitude would have to be larger than[21,22] the colour-allowed tree amplitude (Fig. 1a), while maintaining the large value of $\mathcal{A}_{K^{\pm}\pi^{\mp}}$. The electroweak penguin diagram of Fig. 1d violates isospin, and so might be suspected as a source of the asymmetry. In the standard model, this diagram has a negligible CP violating phase, and cannot affect $\Delta\mathcal{A}$ by much. However, as a loop amplitude, it can pick up a CP violating phase from new physics. If the electroweak penguin explains the effect, this would indicate new physics beyond the standard model[20–22].

A more detailed theoretical calculation[23] indeed supports an enhancement of the colour-suppressed tree contribution, but not to the extent of overpowering the colour-allowed tree contribution. Dominance of the colour-suppressed tree contribution over the colour-allowed tree contribution, though possible from the data, would indicate a breakdown of our theoretical understanding. It could also exacerbate[23] another puzzle arising in related B decays. Mixing-dependent CP violation in $B^0\rightarrow J/\psi K^0$ decay has been measured precisely[3,4]. Similar measurements have been performed on B^0 decays to charmless final states dominated by penguin diagrams analogous to Fig. 1b, such as $B^0\rightarrow K^0\pi^0$. Although the experimental errors are still large, the average value[24] over all penguin dominated modes is 2.5σ smaller than the value from $B^0\rightarrow J/\psi K^0$. In fact, almost all measurements of penguin dominated modes give values of CP violation that are below the value found in the $B^0\rightarrow J/\psi K^0$ mode. This negative deviation, in contrast to theoretical calculations that suggest[25,26] a slightly positive deviation within the standard model, is called the ΔS puzzle. At present there is no theory within the standard model

context that can explain both the ΔS puzzle and the difference between $\mathcal{A}_{K^\pm\pi^\mp}$ and $\mathcal{A}_{K^\pm\pi^0}$. In a specific new physics model and using a detailed calculation, it has been shown[27] that approaching the difference between $\mathcal{A}_{K^\pm\pi^\mp}$ and $\mathcal{A}_{K^\pm\pi^0}$ with new physics in the electroweak penguin can generate a negative ΔS.

More data are needed to confirm the ΔS problem. To further examine new physics effects, one can measure the direct CP asymmetry in $B^0\to K^0\pi^0$ and compare[28,29] it with $\mathcal{A}_{K^\pm\pi^\mp}$ and $\mathcal{A}_{K^\pm\pi^0}$. However, this requires even more data because of the lower detection efficiency for $B^0\to K^0\pi^0$. The precise measurement of mixing-induced and direct CP violation asymmetries is clearly a promising approach to search for new physics, and a major emphasis for the Belle experiment and other future B physics facilities.

In summary, we have measured the CP asymmetries for $B\to K^\pm\pi^\mp$, $K^\pm\pi^0$ and $\pi^\pm\pi^0$ using 535 million $B\bar{B}$ pairs. Direct CP violation in $B^\pm\to K^\pm\pi^\mp$ is observed, accompanied by a large deviation between $\mathcal{A}_{K^\pm\pi^\mp}$ and $\mathcal{A}_{K^\pm\pi^0}$. Although this deviation could be due to our limited understanding of the strong interaction, the difference in direct CP asymmetries for charged versus neutral B decays may be an indication of new sources of CP violation beyond the standard model of particle physics.

METHODS SUMMARY

Charged kaons, charged pions and neutral pions are reconstructed using the information provided by the Belle detector. The dominant $e^+e^-\to q\bar{q}$ background is suppressed by employing event shape variables and B flavour tagging information. CP-violating asymmetries are extracted by performing an unbinned maximum-likelihood fit to the $M_{\rm bc}-\Delta E$ distribution.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 12 July 2007; accepted 7 February 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature

Acknowledgements We thank the KEKB group for the operation of the accelerator, the KEK Cryogenics group for the operation of the solenoid, and the KEK computer group and the NII for computing and Super-SINET network support. We acknowledge support from MEXT and JSPS (Japan); ARC and DEST (Australia); NSFC and KIP of CAS (China); DST (India); MOEHRD, KOSEF and KRF (Korea); KBN (Poland); MES and RFAAE (Russia); ARRS (Slovenia); SNSF (Switzerland); NSC and MOE (Taiwan); and the DOE (USA).

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LETTERS

Super-chondritic Sm/Nd ratios in Mars, the Earth and the Moon

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Small isotopic differences in the atomic abundance of neodymium-142 (^{142}Nd) in silicate rocks represent the time-averaged effect of decay of formerly live samarium-146 (^{146}Sm) and provide constraints on the timescales and mechanisms by which planetary mantles first differentiated[1–4]. This chronology, however, assumes that the composition of the total planet is identical to that of primitive undifferentiated meteorites called chondrites. The difference in the ^{142}Nd/^{144}Nd ratio between chondrites and terrestrial samples may therefore indicate very early isolation (<30 Myr from the formation of the Solar System) of the upper mantle or a slightly non-chondritic bulk Earth composition[5,6]. Here we present high-precision ^{142}Nd data for 16 martian meteorites and show that Mars also has a non-chondritic composition. Meteorites belonging to the shergottite subgroup define a planetary isochron yielding an age of differentiation of 40 ± 18 Myr for the martian mantle. This isochron does not pass through the chondritic reference value ($100 \times \varepsilon^{142}\text{Nd} = -21 \pm 3$; $^{147}\text{Sm}/^{144}\text{Nd} = 0.1966$)[6]. The Earth, Moon and Mars all seem to have accreted in a portion of the inner Solar System with ~5 per cent higher Sm/Nd ratios than material accreted in the asteroid belt. Such chemical heterogeneities may have arisen from sorting of nebular solids or from impact erosion of crustal reservoirs in planetary precursors. The ^{143}Nd composition of the primitive mantle so defined by ^{142}Nd is strikingly similar to the putative endmember component 'FOZO' characterized by high ^{3}He/^{4}He ratios[7,8].

Samarium possesses two radioactive isotopes: ^{147}Sm (half-life of 106 Gyr) and ^{146}Sm (half-life of 103 Myr), which decay to ^{143}Nd and ^{142}Nd respectively. Samarium-146 chronometry is a high-precision tool for dating mantle differentiation in planetary bodies during or shortly after their accretion. As with the long-lived ^{147}Sm–^{143}Nd system, the ^{146}Sm–^{142}Nd chronometer provides a time-integrated record of rare-earth element fractionation. Such fractionation can occur during partial melting or crystallization of silicate reservoirs. But because of its short half-life, and the low initial abundance of ^{146}Sm (^{146}Sm/^{144}Sm = 0.008 at 4.566 Gyr ago)[9], mantle–crust differentiation processes can create significant ^{142}Nd heterogeneity only if they occurred before 4.2 Gyr ago. As a consequence, the magnitude of ^{142}Nd anomalies ($100 \times \varepsilon^{142}$Nd) in planetary material rarely exceeds 50 and, although pioneering work on the subject was initiated in the early 1990s[1], more extensive application of the ^{146}Sm–^{142}Nd system has only been made possible by recent advances in thermal-ionization mass spectrometry[3].

High-precision analyses of meteorites have shown that chondrites have negative ^{142}Nd anomalies compared with terrestrial rocks[5,6]. Anomalies in ordinary chondrites average −20, whereas carbonaceous chondrites have more negative anomalies ranging between −40 and −20. This difference has been attributed to the presence of presolar components in carbonaceous chondrites as inferred from anomalous compositions for barium[6,10], samarium[6,11] and neodymium[6]. When corrected for these nucleosynthetic effects, ordinary and carbonaceous chondrites yield a homogeneous ^{142}Nd composition of $100 \times \varepsilon^{142}\text{Nd} = -21 \pm 3$ (ref. 6). This has been considered to define the composition of the Solar System, and has been used as a reference for modelling the evolution of the Earth. Boyet and Carlson[5] proposed that the higher ^{142}Nd/^{144}Nd ratio of terrestrial samples reflects an early (>4.53 Gyr) episode of crustal extraction followed by recycling of this enriched reservoir in the deep mantle. This hypothesis is supported by the fact that some Archaean rocks have more radiogenic ^{142}Nd/^{144}Nd than modern mantle[3,12], confirming that a crust formed shortly after the Earth's accretion[13]. However, heterogeneities observed in Archaean mantle indicate that this early crust was progressively remixed with its complementary depleted mantle 3.8 Gyr ago[12]. It is also unlikely that this crustal reservoir could have remained stored at the core–mantle boundary without experiencing re-entrainment in the convective mantle[14]. In fact, an extensive search for ^{142}Nd effects in oceanic basalts[12,15,16] has failed to detect negative ^{142}Nd anomalies that would be associated with an ancient crustal component. The alternative possibility, that the terrestrial planets formed from material that differs from all the chondritic meteorites studied so far, must therefore be considered.

Because Mars has preserved the largest ^{142}Nd effects of all known planetary bodies[2], examination of ^{142}Nd data in martian meteorites should provide a crucial test for interpreting terrestrial and chondritic data. Here we present high-precision ^{142}Nd analyses for a large set of martian meteorites. We show that shergottites define a planetary isochron resulting from a mantle differentiation event near the end of the accretion of Mars. We then compare our results with ^{142}Nd data obtained from other planetary bodies and chondritic meteorites.

Whereas the Earth's homogeneous ^{142}Nd/^{144}Nd ratio[5,12] reflects a long geological history of recycling and mixing, the Moon, Mars and Vesta (assumed to be the eucrite parent body) display significant heterogeneities, resulting from the preservation of early differentiated silicate reservoirs[2,4,5]. For these planetary bodies, coupled ^{146,147}Sm–^{142,143}Nd systematics can be used to estimate an age of differentiation using the isochron method. A planetary isochron is obtained by plotting ε^{142}Nd values from mantle-derived samples against the Sm/Nd ratio of their respective source reservoirs. The slope of the isochron yields the age of differentiation of the mantle–crust system. The method can only be applied if both Sm–Nd chronometers were affected by the same episode of differentiation. In the case of the Earth, this condition is not satisfied as ^{147}Sm–^{143}Nd primarily records the extraction of continental crust, whereas ^{146}Sm–^{142}Nd chronometry records an earlier event (>4.45 Gyr ago)[3]. In contrast, long-lived isotopic systems in martian meteorites record an episode of mantle depletion ~4.5 Gyr ago[17].

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This provides strong support for combining ^{147}Sm–^{143}Nd and ^{146}Sm–^{142}Nd in a two-stage model of isotopic evolution.

We analysed 16 martian meteorites for ^{146}Sm–^{142}Nd and ^{147}Sm–^{143}Nd using previously described analytical techniques[12]. The $^{142}Ce/^{142}Nd$ and $^{144}Sm/^{144}Nd$ ratios were reduced to <10 p.p.m. and <1 p.p.m. respectively, such that interference corrections were minimal. During the course of this study, analyses of martian samples were interspersed with analyses of a terrestrial standard (IPGP Ames), which yielded a long-term reproducibility of 6 p.p.m. (2 s.d.) (see Supplementary Table 1). This represents an improvement by a factor of ~3 compared with previous studies of martian samples[2,18–20].

A correlation is obtained for shergottites in a diagram of $\varepsilon^{142}Nd$ versus $^{147}Sm/^{144}Nd$ (Supplementary Table 2 and Fig. 1a). If interpreted as a planetary isochron, this correlation defines an age of mantle differentiation of 40 ± 18 Myr after the beginning of the Solar System. Nakhlites and the Chassigny meteorite (the NC group), however, do not plot on this isochron, and any attempt to calculate an age of differentiation including the NC group yields an age older than the Solar System. Interestingly, the NC group differs from the most radiogenic subgroup of shergottites only with regard to their $^{143}Nd/^{144}Nd$ ratio, having indistinguishable $^{142}Nd/^{144}Nd$ ratios. This provides evidence that the NC mantle was disturbed at a later time, affecting the ^{147}Sm–^{143}Nd system when ^{146}Sm had already completely decayed. In contrast, the model age obtained from shergottites is consistent with the presence of $\varepsilon^{182}W$ anomalies in these meteorites[18,21], because these can only have been generated by very early differentiation (>4.5 Gyr ago).

Our chronological results are comparable to those obtained by Foley *et al.*[18] and Debaille *et al.*[22] using multi-collector inductively coupled plasma mass spectrometry and thermal-ionization mass spectrometry, respectively. Our new data confirm the magnitude of the negative anomalies reported in ref. 16 for the meteorites Shergotty and Zagami but barely overlap with the results for DaG476 and SaU008/SaU005 (reported[18] at 81 ± 26 and 91 ± 31, compared with $\sim 60 \pm 5$ in this study). This discrepancy cannot be attributed to terrestrial contamination, as the addition of crustal Nd would also have lowered the $^{143}Nd/^{144}Nd$ ratio in our samples[19]. Thus, the difference in $^{142}Nd/^{144}Nd$ is probably related to the higher precision attainable for Nd isotopes using new-generation thermal-ionization mass spectrometry. In consequence, the isochron obtained by Foley *et al.*[18] intersects the canonical chondritic Sm/Nd ratio at $\varepsilon^{142}Nd \approx 0$, whereas the isochron reported here has a significantly lower intercept (Fig. 1a). This has important implications for the definition of the bulk composition of Mars and other terrestrial planets, which are further discussed below.

If all terrestrial planets formed from material with identical composition for refractory elements, then planetary isochrons for Mars, the Moon and Vesta should have a common intersection. This should be at $100 \times \varepsilon^{142}Nd = -21 \pm 3$ and $^{147}Sm/^{144}Nd = 0.1966$ if these planetary objects formed from chondritic material[6]. Examination of the ^{142}Nd data, however, leads to very different conclusions. The most striking characteristic of martian meteorites is that only the least radiogenic samples have $\varepsilon^{142}Nd$ as low as ordinary chondrites. These shergottites cannot be representative of the bulk ^{142}Nd composition of Mars, as their mantle source is also characterized by

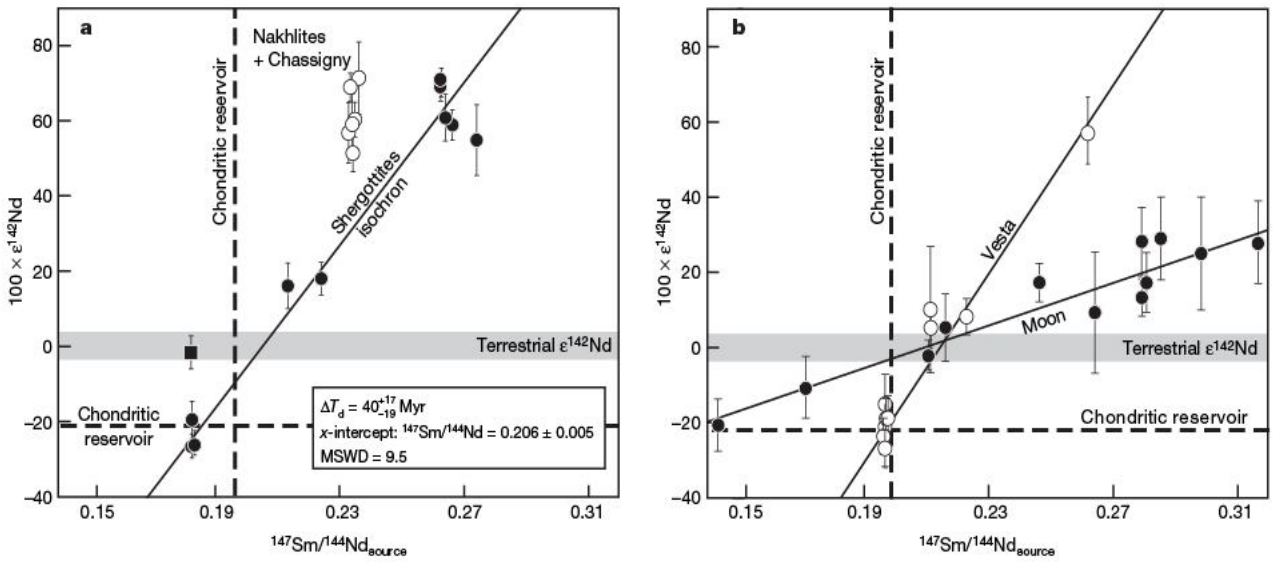

Figure 1 | The $\varepsilon^{142}Nd$ signatures of martian meteorites, lunar samples and eucrites compared with the composition of chondrites and terrestrial samples. a, Values of $\varepsilon^{142}Nd$ for martian meteorites versus Sm/Nd ratios of their source reservoirs. $\varepsilon^{142}Nd$ is defined as $\left(\frac{(^{142}Nd/^{144}Nd)_{sample}}{(^{142}Nd/^{144}Nd)_{std}} - 1\right) \times 10^4$ where std is the terrestrial standard Ames. The ratio $^{147}Sm/^{144}Nd_{source}$ is calculated using the $^{143}Nd/^{144}Nd$ ratio of each sample in the context of a two-stage model. The first stage represents the evolution of a bulk silicate Mars with $^{147}Sm/^{144}Nd \approx 0.2$. Mantle–crust differentiation proceeds instantaneously at $t = T_d$ and differentiated reservoirs evolve without further interaction until the present day. $\left(\frac{^{147}Sm}{^{144}Nd}\right)_{source} = \frac{(^{143}Nd/^{144}Nd)^{S}_{T_s} - (^{143}Nd/^{144}Nd)^{B}_{T_d}}{e^{\lambda T_d} - e^{\lambda T_s}}$, where superscripts S and B stand for sample and bulk Mars respectively, λ is the decay constant of ^{147}Sm ($6.54\ Gyr^{-1}$), T_s is the age of the sample and T_d is the age of mantle differentiation. The ratio $(^{143}Nd/^{144}Nd)^{B}_{T_d}$ is calculated using as reference the chondritic uniform reservoir (CHUR)[26]: $(^{147}Sm/^{144}Nd)_0^{CHUR} = 0.1966$, $(^{143}Nd/^{144}Nd)_0^{CHUR} = 0.512638$, where the subscript 0 stands for present-day. $(^{147}Sm/^{144}Nd)^0_{source}$ is calculated for each sample using an *a priori* value of $T_d = 4.566$ Gyr. An age of differentiation is then calculated using least square regression and this new estimate is used to obtain a new model age. This iterative calculation is repeated until a constant value of T_d is obtained. With the exception of NWA1183 (filled square), all shergottites (filled circles) define an isochron whose slope yields an age of differentiation of 40 ± 18 Myr (2 s.d.). All nakhlites (open circles) and Chassigny have a homogeneous ^{142}Nd signature, consistent with the cogenetic formation of these rocks 1.3 Gyr ago. Nakhlites and Chassigny, however, do not plot on the shergottite isochron, suggesting that a more complex model is required to describe the isotopic evolution of the NC group. **b**, The ^{146}Sm–^{142}Nd systematics of lunar samples[4,25] (filled circles) and eucrites[5] (open circles). Lunar samples with neutron correction higher than 5 p.p.m. were excluded from the regression to avoid potential biases due to different correction methods. The lunar data selected from the databases of Nyquist *et al.*[4] and Boyet and Carlson[25] yield an age of differentiation for the lunar mantle of 239^{+35}_{-45} Myr (mean standard weighted deviation, MSWD = 2.3). Regression of ^{142}Nd data indicates that the Moon must have a bulk $^{142}Nd/^{144}Nd$ ratio close or identical to that of the Earth, whereas eucrite data suggest a chondritic composition for Vesta. Error bars represent the internal precision of individual measurements (2 s.d.).

subchondritic $^{143}Nd/^{144}Nd$. This observation is hard to reconcile with a hidden reservoir model similar to that proposed for the Earth[5]. Indeed, the formation of a crust ~4.52 Gyr ago would shift the position of other reservoirs along the isochron but would not affect the position of the isochron with regard to the chondritic composition. One would need to call on a more complex scheme, whereby a very early episode of mantle depletion would have moved the mantle $^{142}Nd/^{144}Nd$ ratio before differentiation of the shergottite sources. This, however, would require that the first event occurred within a few million years after formation of the Solar System. Such an *ad hoc* model is almost impossible to reconcile with the accretion history of Mars as it requires the segregation of a crust before core formation[23] and crystallization of the martian magma ocean[19].

Debaille *et al.*[22] argued that if Mars had a non-chondritic composition similar to that of the Earth, then the Martian isochron should intersect the terrestrial upper mantle composition at $\varepsilon^{142}Nd = 0$, $^{147}Sm/^{144}Nd \approx 0.23$. Because the mid-ocean ridge basalt source plots off to the right of the shergottite correlation, they argued that shergottites are inconsistent with a non-chondritic composition for Mars and favour a more complex multi-stage mixing model to explain the shift between shergottites and chondrites. But the mid-ocean ridge basalt source is not a plausible composition for the bulk Earth. In fact, a value of $\varepsilon^{142}Nd = 0$ would correspond to a terrestrial $^{147}Sm/^{144}Nd$ ratio lower than that of the upper mantle (~0.21). This bulk Earth composition would plot on the shergottite isochron, which is perfectly consistent with the idea that both planets accreted from non-chondritic material. Boyet and Carlson[5] pointed out that eucrites are not inconsistent with a chondritic composition for Vesta (Fig. 2b). However, reconciling the martian data with a chondritic composition would necessarily require multiple episodes of differentiation. A simpler explanation is that Mars accreted from material with super-chondritic Sm/Nd ratio.

Examination of lunar data indicates that the $^{142}Nd/^{144}Nd$ ratio of the Earth may also reflect a non-chondritic bulk composition rather than internal differentiation. Early studies of lunar samples yielded a young age of crystallization for the Moon (~4.32 ± 0.05 Gyr) and a bulk $\varepsilon^{142}Nd$ value indistinguishable from the terrestrial value[4] (Fig. 1b). These results were disputed by Rankenburg *et al.*[24], who reported low $^{142}Nd/^{144}Nd$ ratios in a series of lunar samples, and proposed a chondritic composition model for the Moon. However, the low $^{142}Nd/^{144}Nd$ ratios measured by these authors in lunar basalts have not been confirmed by a more recent study[25] reporting high-precision results in remarkable agreement with the earlier super-chondritic $\varepsilon^{142}Nd$ values[4]. This discrepancy cannot be explained by the different method used by Rankenburg *et al.*[24] to correct neutron capture effects on the $^{142}Nd/^{144}Nd$ ratio. Indeed, neutron corrections remain negligible (1–5 p.p.m.) for a large majority of samples characterized by short exposure ages[25]. Thus, with the exception of Rankenburg *et al.*[24], studies of lunar samples consistently report $\varepsilon^{142}Nd$ values higher than chondritic[4,25], which could rule out a chondritic composition for the Moon. As illustrated in Fig. 1b, the combined data sets from refs 4 and 25 define an isochron which intersects the shergottite isochron at a value of Sm/Nd ~5% higher than chondritic[26], and a value of $\varepsilon^{142}Nd$ indistinguishable from the terrestrial value. If interpreted in terms of planetary differentiation, this would require the existence of hidden reservoirs on Mars, the Earth and the Moon, which would have shifted the composition of the depleted reservoirs by the same amount. Given the differences in size, age and mineralogy, this seems highly unlikely. The fact that the lunar and martian isochrons intersect at a $^{142}Nd/^{144}Nd$ ratio identical to the terrestrial value can hardly be coincidental. The most straightforward explanation is that all three planetary bodies accreted from material with a Sm/Nd ratio ~5% higher than the composition of chondrites.

We attempted to define a common bulk planetary composition using the intersect of the shergottite isochron with the terrestrial $\varepsilon^{142}Nd$ value (Fig. 2a). This hypothetical composition ($^{147}Sm/^{144}Nd \approx 0.206 \pm 0.005$; $\varepsilon^{142}Nd = 0$) plots within errors on a 4.566-Gyr isochron passing through the chondritic composition (Fig. 2b). If interpreted in terms of internal differentiation, this would require that all the putative hidden reservoirs formed at the very beginning of the Solar System, which seems irreconcilable with the timescale of accretion of the terrestrial planets. The results from Fig. 2b strongly suggest that ^{142}Nd differences between the Earth, Mars and chondrites reflect chemical fractionation in the accretion disk rather than planetary differentiation processes or nucleosynthetic anomalies. Fractionation of Sm/Nd could have arisen from sorting of particles (for example, condensates or chondrules; see Supplementary Fig. 1) in the accretion disk[27,28], resulting in a higher Sm/Nd ratio in the region of formation of the terrestrial planets. Alternatively, high Sm/Nd may have developed in Mars and the Earth as a result of collisional erosion of planetary crusts[29]. This could have produced non-chondritic compositions in large planets by removing reservoirs with low Sm/Nd. Given the large core/mantle ratio of Mercury, it is likely that this planet has had a similar history.

A primitive mantle with $^{147}Sm/^{144}Nd \approx 0.206 \pm 0.05$ would develop an $\varepsilon^{143}Nd$ value of +5 above the chondritic value. A ratio of $^{87}Sr/^{86}Sr = 0.7035$ can then be estimated for the bulk Earth from the Sr–Nd mantle array. This composition is similar to the $\varepsilon^{143}Nd$ signatures observed in oceanic island basalts, which typically range

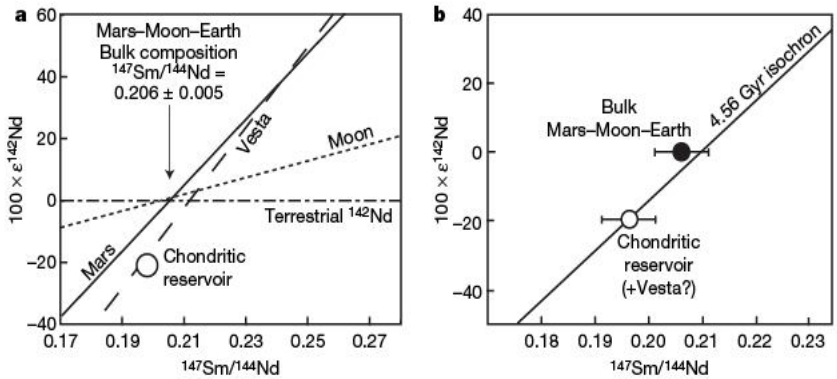

Figure 2 | Planetary isochrons for Mars, Vesta[5] and the Moon[4] compared with terrestrial[3,12] and chondritic compositions[5,6]. **a**, The isochron defined by eucrites intersects the canonical chondritic composition at $100 \times \varepsilon^{142}Nd \approx -20$, favouring a chondritic model for Vesta[5]. In contrast, the terrestrial, martian and lunar data are inconsistent with a chondritic composition. All three planetary bodies may have a common isotopic and chemical composition, corresponding to $\varepsilon^{142}Nd = 0$ and $^{147}Sm/^{144}Nd \approx 0.206 \pm 0.005$. **b**, The Sm–Nd planetary and meteorite compositions plot within errors on a 4.566-Gyr isochron passing through the chondritic reference value, suggesting that the radiogenic $^{142}Nd/^{144}Nd$ ratio observed in all terrestrial rocks reflects fractionation of rare-earth elements during the earliest stages of planetary accretion. Error bars represent the internal precision of individual measurements (2 s.d.).

between +4 and +6 (ref. 30). This signature is also indistinguishable from FOZO, a putative mantle component common to oceanic basalts worldwide[7,8], which has been argued to represent lower-mantle material based on its high $^{3}He/^{4}He$ ratio, pointing to a more primitive composition[7]. Depletion by continental crust is still required to produce the radiogenic $\varepsilon^{143}Nd$ characterizing the source of mid-ocean ridge basalts. However, the mass fraction of depleted mantle needed to balance the Nd budget of the crust amounts to 60% of the silicate Earth, compared with 25% using chondritic parameters. This new mass balance is inconsistent with a compositional stratification at the 670-km discontinuity.

Received 24 July 2007; accepted 18 January 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank T. Kleine for critical discussion and an informal review of the manuscript, and S. Jacobsen for a constructive review. This study was supported by the CNRS research programme PNP.

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LETTERS

Biodiversity and biogeography of phages in modern stromatolites and thrombolites

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Viruses, and more particularly phages (viruses that infect bacteria), represent one of the most abundant living entities in aquatic and terrestrial environments. The biogeography of phages has only recently been investigated and so far reveals a cosmopolitan distribution of phage genetic material (or genotypes)[1–4]. Here we address this cosmopolitan distribution through the analysis of phage communities in modern microbialites, the living representatives of one of the most ancient life forms on Earth. On the basis of a comparative metagenomic analysis of viral communities associated with marine (Highborne Cay, Bahamas) and freshwater (Pozas Azules II and Rio Mesquites, Mexico) microbialites, we show that some phage genotypes are geographically restricted. The high percentage of unknown sequences recovered from the three metagenomes (>97%), the low percentage similarities with sequences from other environmental viral ($n = 42$) and microbial ($n = 36$) metagenomes, and the absence of viral genotypes shared among microbialites indicate that viruses are genetically unique in these environments. Identifiable sequences in the Highborne Cay metagenome were dominated by single-stranded DNA microphages that were not detected in any other samples examined, including sea water, fresh water, sediment, terrestrial, extreme, metazoan-associated and marine microbial mats. Finally, a marine signature was present in the phage community of the Pozas Azules II microbialites, even though this environment has not been in contact with the ocean for tens of millions of years. Taken together, these results prove that viruses in modern microbialites display biogeographical variability and suggest that they may be derived from an ancient community.

Microbialites are organosedimentary structures accreted by sediment trapping, binding and *in situ* precipitation due to the growth and metabolic activities of microorganisms[5]. Stromatolites and thrombolites are morphological types of microbialites classified by their internal mesostructure: layered and clotted, respectively[5]. Microbialites first appeared in the geological record ~3.5 billion years ago, and for more than 2 billion years they are the main evidence of life on Earth[6,7]. Whether modern microbialites are proxies of ancient ecosystems is a major outstanding question[6].

Viruses, and more specifically phages, are the most abundant biological entities in the world's oceans[8]. Phages influence microbial growth rates, genetic exchange, diversity and adaptation, and thus evolution[8]. Current biogeographical studies of phages suggest that they are cosmopolitan in distribution, unlike some examples of highly endemic populations of bacteria and archaea[9–12]. Metagenomic analysis of viral communities from four major ocean regions using the same pyrosequencing technology has shown that essentially all marine viruses are spread widely throughout the oceans[1]. Identical phage-encoded exotoxin genes, T7-like DNA polymerase genes and T4-like structural genes are found in disparate terrestrial, aquatic and extreme environments[2–4]. Phages from soil, sediments and fresh water can productively infect marine microbes[13,14], showing that viruses move between major biomes.

Our metagenomic analysis of viral communities associated with a marine stromatolite (Highborne Cay, Bahamas) and two neighbouring (30 km) freshwater thrombolites and stromatolites (Pozas Azules II and Rio Mesquites, Mexico; Supplementary Fig. 1) showed that most of the sequences (98.8, 99.3 and 97.7% for Highborne Cay, Pozas Azules and Rio Mesquites, respectively) were unique when compared with the sequences in the non-redundant GenBank/SEED databases (BLASTx, E-value $<10^{-2}$). This proportion is much higher than any other previously sequenced viral metagenome (70–90% unknowns[1,15]). A comparison of microbialite metagenomic sequences with 42 viral and 36 microbial metagenomic libraries generated using the same pyrosequencing technology (Tables 1 and 2, respectively; Supplementary Tables 1 and 2 for details), showed that they were less than 5% similar (BLASTn, E-value $<10^{-3}$), further confirming that these are largely unrelated viral communities.

Using the approach developed by Angly *et al.*[1], random subsets of 10,000 sequences from each virome were assembled against each other to identify cross-contigs (that is, sequence overlaps between two samples). A read from one metagenome that assembled with a read from another metagenome indicated an overlap between these two metagenomes[1]. Only contigs produced by sequences from different metagenomes were taken into account to assess how many species were common to the two communities (percentage shared)[1]. Comparisons between Highborne Cay and Pozas Azules II and between Highborne Cay and Rio Mesquites did not produce any cross-contigs, indicating that none of the viruses was shared between these microbialites. The Pozas Azules II-Rio Mesquites comparison produced a very small average cross-contig spectrum, again indicating that essentially nothing is shared between these samples, even though they were taken from microbialites located 30 km from each other. A Monte Carlo analysis of the cross-contig spectra showed that the percentage of genome shared between Pozas Azules II, Highborne

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Table 1 | Similarity among the microbialite viral metagenomes and other environmental viral metagenomes

	Average percentage similarity (BLASTn, E-value $<10^{-3}$)*		
	Highborne Cay viral metagenome	Pozas Azules II viral metagenome	Rio Mesquites viral metagenome
Highborne Cay	100	1.140	0.910
Pozas Azules II	4.020	100	1.100
Rio Mesquites	0.970	0.700	100
Freshwaters ($n = 4$)	1.154 ± 0.240	0.477 ± 0.031	0.916 ± 0.278
Coral reef waters ($n = 4$)	1.462 ± 0.285	0.840 ± 0.032	0.808 ± 0.043
Marine waters ($n = 4$)	1.770 ± 0.573	0.585 ± 0.116	0.543 ± 0.098
Fish ($n = 4$)	0.701 ± 0.156	0.279 ± 0.015	0.387 ± 0.061
Mosquito ($n = 1$)	0.731	0.273	0.683
Coral ($n = 6$)	0.735 ± 0.150	0.290 ± 0.027	0.243 ± 0.024
Human ($n = 2$)	0.881 ± 0.336	0.377 ± 0.019	0.375 ± 0.019
Saltern waters ($n = 11$)	0.690 ± 0.145	0.439 ± 0.059	0.445 ± 0.058
Marine sediments ($n = 3$)	0.654 ± 0.079	0.568 ± 0.057	0.401 ± 0.089

* Average percentage similarity ± s.e.m.

Table 2 | Similarity among the microbialite viral metagenomes and other environmental microbial metagenomes

	Average percentage similarity (BLASTn, E-value $<10^{-3}$)*		
	Highborne Cay viral metagenome	Pozas Azules II viral metagenome	Rio Mesquites viral metagenome
Highborne Cay	47.104	0.400	0.230
Pozas Azules II	4.310	3.742	0.410
Rio Mesquites	1.021	0.637	0.541
Freshwaters ($n = 4$)	1.853 ± 0.609	0.466 ± 0.083	0.559 ± 0.091
Coral reef waters ($n = 4$)	0.903 ± 0.256	0.340 ± 0.050	0.276 ± 0.022
Fish ($n = 4$)	0.288 ± 0.015	0.252 ± 0.007	0.331 ± 0.038
Coral ($n = 7$)	0.805 ± 0.167	0.255 ± 0.016	0.252 ± 0.031
Saltern waters ($n = 11$)	0.655 ± 0.122	0.419 ± 0.034	0.398 ± 0.037
Subterranean ($n = 2$)	0.959 ± 0.377	0.442 ± 0.045	0.470 ± 0.122
Marine sediments ($n = 1$)	1.168	0.432	0.321

* Average percentage similarity ± s.e.m.

Cay and Rio Mesquites was zero (Supplementary Fig. 5) and therefore that the viruses are genetically unique in all three microbialites.

The small number of 'known' phage sequences in the microbialite metagenomes was assigned taxonomical designations based on the top BLAST similarities (Fig. 1, right panel). Their relative abundances were plotted onto the Phage Proteomic Tree[16] (PPT; Fig. 1, left panel). Microphages (icosahedral single-stranded DNA phages infecting *Escherichia coli, Bdellovibrio, Chlamydia* and *Spiroplasma* species[17], Supplementary Fig. 3) were the most common phages in the Highborne Cay and Pozas Azules II phage communities, representing 93.1% and 13.5% of the known phage sequences, respectively. In contrast, microphages were absent in Rio Mesquites, and the phage community was dominated by *Shewanella oneidensis* prophages (MuSo2 and LambdaSo) and *Burkholderia cepacia* phage sequences (54.6% of the total number of phage reads). At the taxonomic resolution of the PPT, the Highborne Cay and Pozas Azules II viral communities resembled each other and a previously described marine virome from the Sargasso Sea, which also contained high

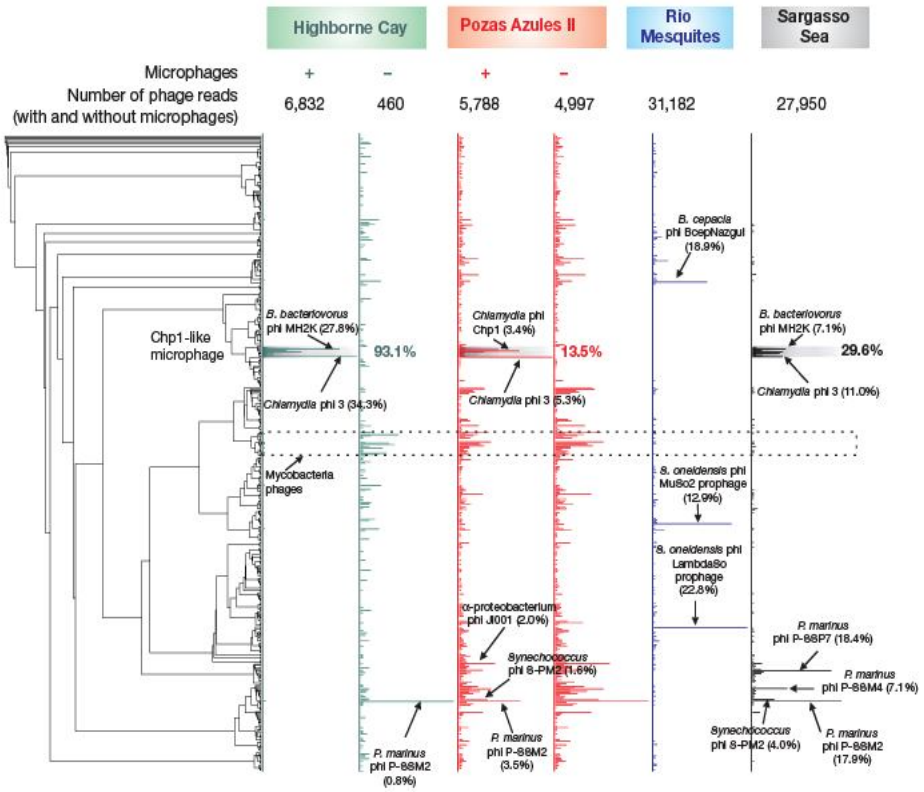

Figure 1 | The phage proteomic tree. The tree (left) shows the similarities of the viral metagenomic sequences to completely sequenced phage genomes. The presence and abundance of phage reads (right; abundance is proportional to line length) are presented in green for Highborne Cay, red for Pozas Azules II, blue for Rio Mesquites and grey for the Sargasso Sea samples. The total number of reads with significant similarity to phages (plus and minus microphages) is also indicated for Highborne Cay and Pozas Azules II. The name of the phage associated with the most abundant reads of each metagenome is given as well as the percentage of the total represented by these reads.

abundances of microphages (29.6%), *Prochlorococcus* phages P-SSM2 and P-SSM4 and *Synechococcus* phage S-PM2 (ref. 1) (Fig. 1).

Genetic distances of the microphages in Highborne Cay, Pozas Azules II and the Sargasso Sea were calculated using global alignments of the viral capsid protein (Vp1) reconstructed from the metagenomes (Fig. 2). The microphages from these three environments clustered together and were branched to the group of phages infecting *Chlamydia*. However, cross-assembly of the microphage nucleic-acid sequences did not produce a single cross-contig, indicating that amino-acid-level functionality is maintained but the nucleic acids have significantly diverged. On the basis of each consensus sequence recovered from the Highborne Cay, Pozas Azules II and Sargasso Sea metagenomes (Supplementary Information part 2), primers targeting the *Vp1* genes were designed (Supplementary Table 4). The capsid genes were successfully amplified from these metagenomes. No polymerase chain reaction (PCR) products were obtained when one sample was tested with the two other primer sets (for example, PCR of Highborne Cay viral DNA with the Pozas Azules II or the Sargasso Sea primer sets). Phylogenetic analysis of PCR products from the Highborne Cay sample showed that the similarity between clones and cultured microphage capsid sequences ranged from 47.5 to 61.2% at the nucleic-acid level and from 37.2 to 69.3% at the protein level, respectively (Supplementary Figs 8A and 8B).

We previously recovered cosmopolitan, essentially identical, T7-like podophage DNA polymerase sequences in the major biomes on Earth, including: marine, freshwater, sediment, terrestrial, extreme and metazoan-associated[3]. These environmental samples, as well as other marine microbial mats from different parts of the world (11 samples—from France, Israel, Bahamas, Puerto Rico and Connecticut, USA), were tested for the presence of the Highborne Cay microphages (Supplementary Table 5). No such microphages were detected in all the environmental samples tested, even though our PCR was sensitive enough to amplify fewer than 100 copies of the *Vp1* gene (Supplementary Fig. 6). New Highborne Cay stromatolite samples (July 2007) tested positive for the presence of the microphages, further confirming that these phages are native to the Highborne Cay stromatolites and persistent across time. To our knowledge, this is the first evidence of endemism in phages.

A 'marine signature' of the microbes from the Cuatro Ciénegas Basin was recently described by Souza *et al.*[18], implying that the whole ecosystem may be derived from an ancient marine community. Similarly, weighted and unweighted Unifrac analyses of the PPT (Supplementary Figs 4A, B) showed a genetic overlap between the Gulf of Mexico, the Sargasso Sea and the Pozas Azules II phage communities, even though these environments have not been in contact since the late Jurassic. This observation supports the hypothesis that phages in modern microbialites may be relicts from an ancient community. An alternative hypothesis that we cannot exclude is that there was a recent marine phage introduction, possibly through aerial vectors such as birds or airborne particles. However, the observation that these microbialite phages are extremely diverged from the global virome and from its nearest neighbour is more congruent with our ancient phage hypothesis.

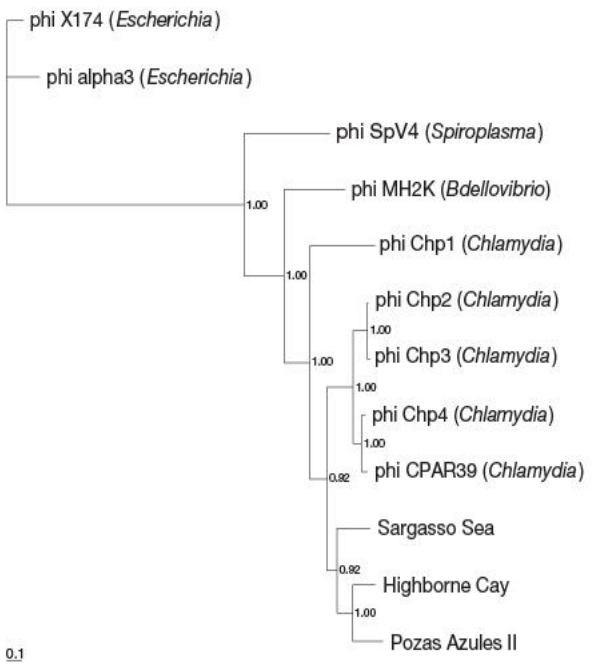

Figure 2 | Phylogenetic relationships among viral capsid amino-acid sequences of microphages. The Bayes values represent the proportion of sampled trees in which those sequences are clustered together.

METHODS SUMMARY

Microbialites were collected from the Pozas Azules II (PAII) pool and the Rio Mesquites (RM) River located in the Cuatro Ciénegas Basin (Mexico) and from the Highborne Cay (HC) marine waters (Bahamas). The viral particles were resuspended and purified using a combination of filtration and caesium chloride density gradient centrifugation[15]. Viral DNA was isolated by a formamide/CTAB extraction[19] and amplified with GenomiPhi (GE Healthcare) following the manufacturer's recommendations. Approximately 10 μg purified DNA was sequenced using pyrosequencing technology[20] (454 Life Sciences).

The sequences from each metagenome were compared to the SEED non-redundant database, our in-house phage database and 78 other metagenomes (using BLAST). The presence and the abundance of the sequences that have the phage databases were mapped onto the PPT (Fig. 1) using Bio-Metamapper (http://scums.sdsu.edu/Mapper). The diversity of the viral community and the percentage of viral genomes shared among samples were determined as previously described[1]. The genetic distances were calculated using the online UniFrac tool[21]. The Isolation by Distance web service[22] was used to test the correlation of the geographical distance and the genetic divergence between two viral communities.

Microphage capsid consensus sequences were reconstructed from the HC, PAII and Sargasso Sea[1] metagenomes and replaced onto a phylogenetic tree (Fig. 2). Primers were designed on the basis of these sequences (Supplementary Table 4) to retrospectively amplify the microphage capsid from the HC stromatolites. These sequences were cloned, sequenced (8 clones) and replaced in phylogenetic trees (Supplementary Figs 8A and 8B). PCR detection limit was defined (Supplementary Fig. 6) and optimal conditions were used to test the occurrence of the HC microphages in 63 different environmental samples (Supplementary Table 5).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 5 December 2007; accepted 23 January 2008.
Published online 2 March 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements Logistical field support was provided by the crew of the RV *Walton Smith*, Highborne Cay management and personnel of the Area de Proteccion de Flora y Fauna of Cuatro Ciénegas. This work was supported by an NSF grant to F.R. Support for B.K.S. and D.L.V. was provided by the NSF. M.B. was supported by a grant from the University of South Florida's Internal New Research Awards Program. V.S. was funded by the CONACYT 2002-C01-0237 project. The authors thank P. Visscher, K. Przekop, L. Rothschild, D. Rogoff, V. Michotey, P. Bonin, S. Norman and E. Bowlin for providing samples of marine microbial mats and M. Schaechter for a critical reading of the manuscript.

Author Contributions C.D. and F.R. designed the project. C.D. analysed most of the bioinformatic results, conducted the molecular biology and wrote the article. S.K. performed the bayesian analysis. S.R. implemented the cross-contig analyses. M.H. extracted viral DNAs. B.R.-B., H.L., F.E.A. and R.A.E. performed bioinformatic analyses. R.V.T. and D.H. helped with the interpretation of the bioinformatic results. V.S., M.B., J.S. and R.P.R. collected the samples. B.K.S., D.L.V., M.F., T.T., L.L., Y.R., L.W. and B.C. provided metagenomic data. F.R. supervised the project and helped with the writing. All authors edited and commented on the manuscript.

Author Information The microbialite viral metagenomes have been deposited into the ftp server of the SEED public database ftp://ftp.theseed.org/metagenomes under the project accession numbers 4440323.3 (Highborne Cay), 4440320.3 (Pozas Azules II) and 4440321.3 (Rio Mesquites). The metagenomes are also publicly accessible in the CAMERA metagenomic database (http://camera.calit2.net) under the project accession numbers HBCStromBahamasVir011105 (Highborne Cay), PAStromCCMexVir072205 (Pozas Azules II), and RMStromCCMexVir072205 (Rio Mesquites). The *Vp1* cloned sequences from the Highborne Cay sample have been deposited in GenBank under accession numbers EF679227 to EF679234. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.D. (cdesnues@yahoo.fr).

LETTERS

Survival variability and population density in fish populations

Coilín Minto[1], Ransom A. Myers[‡] & Wade Blanchard[2]

To understand the processes that regulate the abundance and persistence of wild populations is a fundamental goal of ecology and a prerequisite for the management of living resources. Variable abundance data, however, make the demonstration of regulation processes challenging[1–3]. A previously overlooked aspect in understanding how populations are regulated[4–6] is the possibility that the pattern of variability—its strength as a function of population size—may be more than 'noise', thus revealing much about the characteristics of population regulation. Here we show that patterns in survival variability do provide evidence of regulation through density. Using a large, global compilation of marine, anadromous and freshwater fisheries data, we examine the relationship between the variability of survival and population abundance. The interannual variability in progeny survival increases at low adult abundance in an inversely density-dependent fashion. This pattern is consistent with models in which density dependence enters after the larval stage. The findings are compatible with very simple forms of density dependence: even a linear increase of juvenile mortality with adult density adequately explains the results. The model predictions explain why populations with strong regulation may experience large increases in variability at low densities[7]. Furthermore, the inverse relationship between survival variability and the strength of density dependence has important consequences for fisheries management and recovery, and population persistence or extinction[8–10].

Hitherto, the analysis of population density regulation has focused on the mean response of the per-capita rate of population change over population density; empirically manifested in tests of return tendency in abundance data[11]. This approach has considerably increased our understanding of population dynamics[5] and for many taxa, density-dependent regulation is readily discerned; however, highly variable populations (chiefly insects) can often defy attempts to detect density regulation of abundance[12]. Among highly variable taxa, fish populations have been somewhat neglected in the density regulation literature. In fact, the extreme variability of reproductive success in fish populations (Fig. 1) suggests that they provide ideal data for tests of proposed links between variability and the strength of population regulation[13]. We develop an alternative approach to understanding population regulation by focusing on the variance in survival. Using theoretical exposition and a meta-analysis of 147 wild populations, we demonstrate that survival variability in fish populations has a specific and consistent pattern, increasing with decreasing abundance. Moreover, we show that high variability does not preclude simple density regulation[14]. In the process, we demonstrate the viability of using patterns in the variance rather than the mean response to overcome the general ecological hurdle of interpreting markedly variable data.

Fish populations pass through a number of life-history stages, from egg to larval to juvenile, before joining the adult population. To analyse the effect of density dependence on the relationship between variability and reproductive adult abundance we examine models in which density-dependent mortality arises in the juvenile stage, an approach that has been shown to be suitable for many fish populations[15]. Stochastic mortality, independent of density, is assumed to take place during egg, larval and juvenile stages. Using these assumptions and a suite of commonly applied models for survival ranging from no density dependence (constant productivity) to extreme overcompensation[13] (survival continually declines with increasing abundance), we derive predictions for the relationship between survival variability and population density (see derivations in the Methods and Supplementary Information).

Figure 2 shows the predicted relationships between survival variability and adult abundance under different survival model

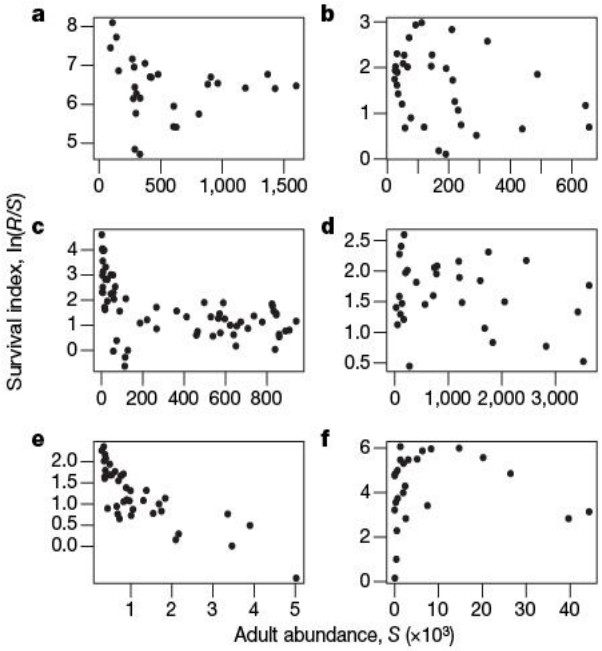

Figure 1 | Example relationships between the survival index and adult abundance. Examples of the Gadidae, Clupeidae and Salmonidae families, chosen to graphically accompany specific points made on the relationship between survival variability and population density. **a**, Cod from Labrador/northeast Newfoundland, Canada. **b**, Silver hake from the Mid-Atlantic Bight. **c**, Herring from Downs stock, North Sea, UK. **d**, Sardine from California, USA. **e**, Atlantic salmon from the Margaree River, Nova Scotia, Canada. **f**, Pink salmon from Sashin Creek, Little Port Walter, Alaska, USA. The greatest variability occurs for populations reduced to very low levels (Downs herring) and Icelandic spring-spawning herring. Extreme variation is shown in the Sashin Creek pink salmon population, for which the highest variation in survival occurs when the number of females spawning was reduced to below 300.

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formulations. In comparison to the density-independent form, all density-dependent models predict marked changes in the variance in survival over adult density; including a general increase in variance at low abundance where the models exhibit compensatory survival (increasing survival). The degree of compensation increases from Fig. 2a through to Fig. 2e. The variance in survival declines monotonically for survival models displaying only compensatory survival. For over-compensatory models where survival continually declines with increasing abundance with no asymptote (for example, the Ricker and Schaefer models in Fig. 2d and e) the variance in survival is predicted to initially decrease, then increase with adult abundance.

Maximum likelihood was used to estimate the parameters of a general Deriso–Schnute[16,17] survival model, assuming that the variance is not constant but follows a functional form of the explanatory variable[18] (adult density S) as in $\sigma^2 = \exp(\eta_0 + \eta_1 S)$ (see Methods). This parameterization enables us to estimate a coefficient of heteroscedasticity η_1, which indicates how much and in which direction the variance is changing over adult density in a given population. We then combine these estimates within and across species in a formal meta-analysis (see Methods).

Figure 3 shows the heteroscedastic coefficient estimates combined across populations by species under three different survival model formulations. There is a consistent trend indicated by both the fixed-effects and overall mixed-effects results for an inverse relationship between the variance in survival and adult abundance (see the individual fits in the Supplementary Information). Species for which there are more than four populations emphasize this point in that the decline in survival variability is generally conserved across different survival model formulations. We describe a general mechanism that can explain the changing variability in survival over adult abundance, density-dependent mortality in the juvenile phase following stochastic density-independent mortality in the egg and larval stages (see Methods).

If density-dependent population regulation decreases the variance in survival with increasing density, why then should we see high variability in strongly regulated populations? Assuming that density-dependent mortality is linear in log-abundance, the variance in survival is given by:

$$\mathrm{Var}(\ln(R_t/S_0)) \approx (1-\lambda)^2\sigma_\varepsilon^2 + \sigma_\delta^2 \quad (1)$$

where R_t and S_t are the number of recruits and the number of spawners at time t, λ is density-dependent juvenile mortality and σ_ε^2 is the variance in mortality in the egg and larval stages and σ_δ^2 is the variance in survival during the juvenile phase unrelated to density (see derivation in the Methods section). We choose the density-dependent juvenile mortality to be $\lambda \approx 0.5$ (ref. 15). This corresponds to very strong population regulation in that a hundredfold increase in the abundance of cod entering the juvenile stage would yield only a tenfold increase in the abundance of cod surviving the juvenile stage[15]. Such strong regulation might suggest that recruitment variability of cod should be weak, but this is not the case: cod populations typically have a standard deviation of log recruitment in the 0.5 to 1.0 range[19]. That recruitment variability is strong, despite regulation, is a consequence of the extremely large variability in larval abundance[20,21]. If the variance in the juvenile mortality unrelated to density is ignored[18] then $\sigma_{\ln(R/S)} = (1-\lambda)\sigma_\varepsilon$. Thus, $\sigma_{\ln(R/S)}$ will be reduced to about half of σ_ε; however, despite this attenuation, the large magnitude of σ_ε ensures that there will be strong survival variability. The key to understanding population regulation in this taxon is that although the observed survival variability may be high, this is the result of highly variable stochastic mortality in the larval phase but where density-dependent regulation occurs in the juvenile phase we observe marked patterns of change in survival variability over adult density (Fig. 3).

Our treatment does not amount to demographic stochasticity[22] alone, where individual fitness variance increases at greatly reduced abundances, accompanying population-level Allee effects. In fact, including depressed survival at very low abundances (depensation) only serves to exacerbate survival variability (Fig. 2f, depensatory Beverton–Holt model) on top of underlying changes across the whole

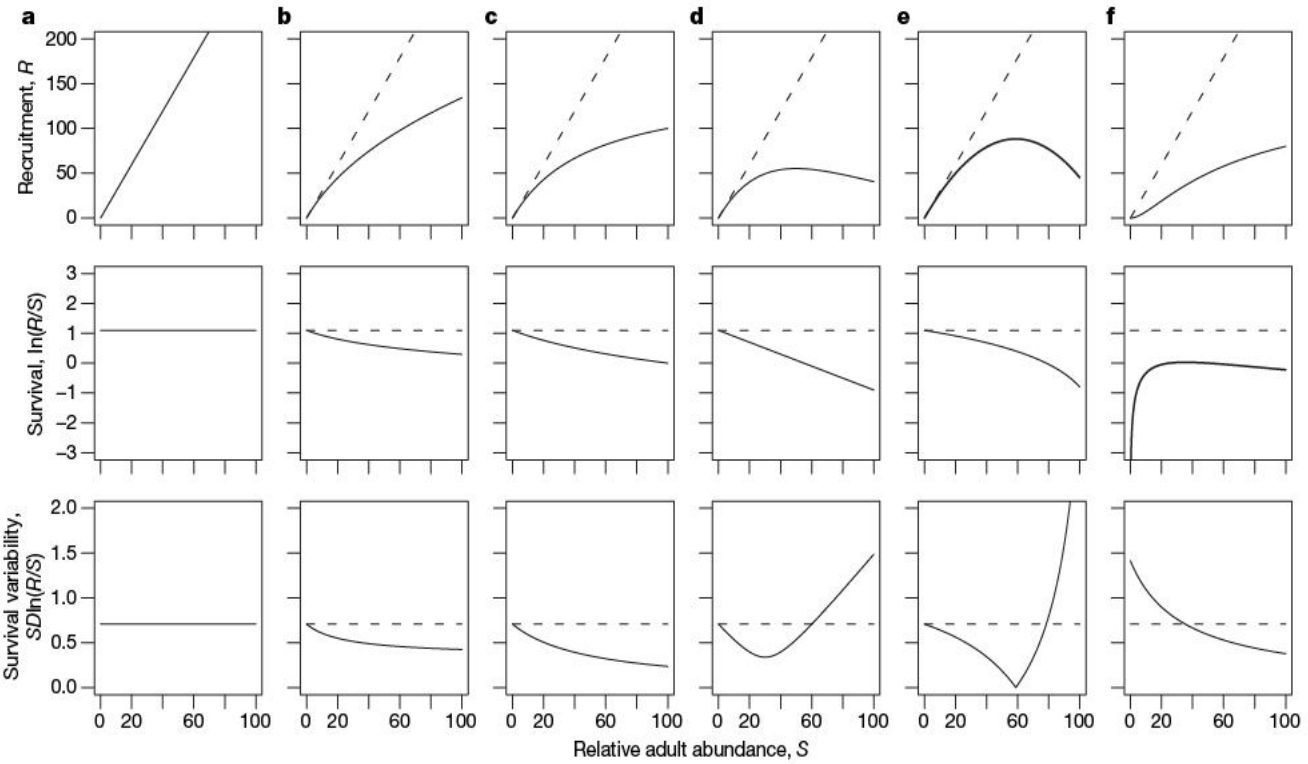

Figure 2 | The predicted relationships between adult abundance and recruitment, survival and survival variability. The models are realizations of the Deriso–Schnute general stock-recruitment model[16,17] with the shape parameter γ corresponding to: $\gamma = -1{,}000$ (**a**, no density dependence), $\gamma = -2$ (**b**, Cushing-like), $\gamma = -1$ (**c**, Beverton–Holt), $\gamma = 0$ (**d**, Ricker), $\gamma = 1$ (**e**, Schaefer) and $\gamma = -1$ (**f**, for the depensatory Beverton–Holt model; see Supplementary Information). The other parameters chosen were $\alpha = 3$ and $\beta = 0.02$ for all models except the Schaefer model, for which $\beta = 0.0085$ (Supplementary Information). The dotted lines are realizations in the absence of density dependence.

range of densities (Fig. 2c, the usual Beverton–Holt model). In addition, using the same data set, the presence of characteristic 'downward hooks' of recruitment over the adult abundance, indicating depensation, is debated[23,24].

Using the commonly applied Ricker and Schaefer models, which exhibit overcompensation (survival continually declines at higher adult abundances), we have shown that density-dependent survival variability will actually increase again at larger adult abundances (Fig. 3). This behaviour is not captured in approaches that predict that demographic variance will affect populations only at low abundances[25]. We have empirically found that the variability in survival is greater at low rather than higher abundances (see individual fits in the Supplementary Information); whereas the predictions for the Ricker and Schaefer models are that the variance should reach a minimum and then increase. This apparent discrepancy is because all the data we have used comes from exploited populations (most very highly exploited), so that we simply do not have data at high population levels[26].

Other mechanisms may be important for some species. Populations reduced to low abundances also have altered age and size structure. As cod populations were reduced by fishing, the reduction in older ages resulted in a reduction in the seasonal duration of spawning[27]. The reduction of the seasonal duration of spawning should increase the variability in survival of eggs and larvae because the probability that larval emergence will coincide with environmentally favourable conditions, such as the peak abundance of zooplankton, will be reduced[21].

In contrast to density-independent random walks, which allow populations to plummet to irrecoverably low densities, density dependence has long been considered a safeguard from population extinction[14]. From a fisheries perspective, survival should be sufficiently high at low densities so as to mitigate the effects of driving the population down. However, the greatest survival variability occurs for populations reduced to very low levels, such as Downs herring in the North Sea and Iceland spring-spawning herring, both of which were greatly overexploited[13].

Extreme variation is exhibited by pink salmon from Sashin Creek, Alaska, where the highest variation in survival occurs when the number of females spawning was reduced to below 300 (Fig. 1). The increased variance at lower adult abundance will result in higher extinction risk not accounted for in current projections[8,9]. An immediately practical implication for recovery is that current biological reference points and recovery projections are based upon the maximum reproductive rate at low population sizes, estimated from the slope of the stock-recruitment function at the origin. Recruitment is currently deemed to be lognormally distributed by assuming that the survival rates in each life-history stage are an independent random variable and the sum of these on the log scale is normally distributed[28]. This would imply that recruitment variability would increase with the mean recruitment and equivalently that survival should be normally distributed at a given abundance with a constant variance.

However, our treatment has shown the variance in survival to be in general non-constant over abundance (Fig. 2). If the maximum reproductive rate is estimated from the data here shown to be naturally heteroscedastic (non-constant variance), then erroneous recovery projections could result. Our model results show that survival variability can be inversely density-dependent in that the steepness of the increase in variability of survival as zero density is approached depends on the strength of the density dependence parameter (sensitivity analysis in Supplementary Information). Populations with very strong density dependence may exhibit greatly increased survival variability during population declines.

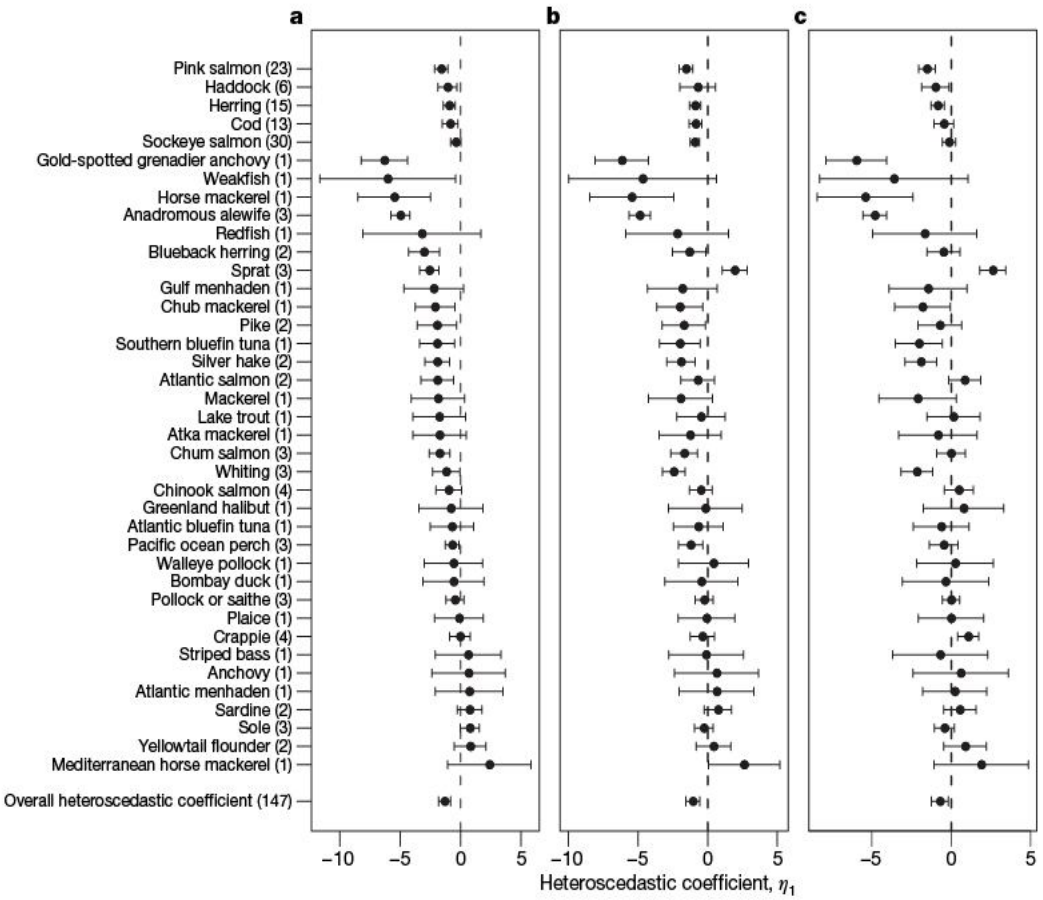

Figure 3 | Estimates of the heteroscedastic coefficient η_1 in survival across available fish species. Survival models: **a**, Schaefer ($\gamma = 1$); **b**, Ricker ($\gamma = 0$); and **c**, Beverton–Holt ($\gamma = -1$). The number of populations per species is given in parentheses and the error bars represent the 95% confidence intervals on the estimate. For species where the number of populations is greater than four, the estimate represents a fixed-effects estimate using all populations simultaneously. For species with four or less populations a weighted average of the individual population estimates is provided. An overall estimate of the heteroscedastic coefficient is provided by a random-effects meta-analysis (Supplementary Information).

Incorporating this heteroscedastic component by weighting will affect estimates of the slope at the origin and thus alter recovery projections for severely depleted populations.

METHODS SUMMARY

Models for the variance in survival. A derivation of the survival variability model is presented in the Methods and fully expanded upon in the accompanying Supplementary Information.

Data. The data come from a standardized global compilation of stock-recruitment data for over 500 species[29]. The data are standardized so that recruits and spawners have the same units[13]. To avoid the subsequent meta-analytical means being dominated by populations with large ranges of adult abundance and thus small standard errors, the recruits and spawners were further standardized to range between 0 and 1. Only data sets with at least 15 pairs of spawner recruit observations and where the ratio of the maximum observed adult abundance to the minimum was at least five were used. This was done to eliminate data sets which had little power to address the question[30] and resulted in the analysis of 147 populations of 39 species.

Likelihood. A log-likelihood function for a regression of survival $\ln(R/S)$ on spawning stock biomass S with normally distributed errors and a fixed survival mean $\mu_i = \ln(\alpha) + \ln(1 - \beta_\gamma S_i)^{1/\gamma}$ from the three-parameter Deriso–Schnute[16,17] stock-recruitment model (see Methods) at a given S_i and variance σ^2 is given by:

$$l(\mu, \sigma^2) \propto -\frac{1}{2}\sum_{i=1}^{n} \ln\sigma^2 - \frac{1}{2}\sum_{i=1}^{n} \frac{\left(\ln\left(\frac{R_i}{S_i}\right) - \mu_i\right)^2}{\sigma^2} \quad (2)$$

To investigate the relationship between survival variability and population density, the variance term can be re-parameterized as a functional form of adult abundance[10,18]. The log-likelihood is now written:

$$l(\mu, \eta_0, \eta_1) \propto -\frac{1}{2}\sum_{i=1}^{n} (\eta_0 + \eta_1 S_i) - \frac{1}{2}\sum_{i=1}^{n} \frac{\left(\ln\left(\frac{R_i}{S_i}\right) - \mu_i\right)^2}{e^{\eta_0 + \eta_1 S_i}} \quad (3)$$

If the variance is constant over adult abundance, the heteroscedastic coefficient $\eta_1 = 0$ and a constant variance is recovered at $\sigma^2 = e^{\eta_0}$.

Meta-analysis. A full description of the fixed and mixed-effects meta-analytical methods, used to estimate the heteroscedastic coefficients is provided in the Supplementary Information.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 18 May; accepted 21 December 2007.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank D. Tittensor for comments on the variance derivations and A. Edwards, B. Worm, D. Ricard and C. Barber for comments on the manuscript. This work was funded partly by the Irish government and partly by the European Union, under the National Development Plan 2000–2006, through "Supporting Measures in the Fisheries Sector" (to C.M.).

Author Contributions The original idea for this study was conceived by R.A.M. R.A.M., C.M. and W.B. developed the theoretical models for the variance in survival. C.M. conducted the empirical analyses. All authors contributed to the writing of the manuscript.

Author Information All data used are available at the stock-recruitment database www.mathstat.dal.ca/~myers/welcome.html. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.M. (mintoc@mathstat.dal.ca).

LETTERS

Winners don't punish

Anna Dreber[1,6]*, David G. Rand[1,2]*, Drew Fudenberg[3] & Martin A. Nowak[1,4,5]

A key aspect of human behaviour is cooperation[1–7]. We tend to help others even if costs are involved. We are more likely to help when the costs are small and the benefits for the other person significant. Cooperation leads to a tension between what is best for the individual and what is best for the group. A group does better if everyone cooperates, but each individual is tempted to defect. Recently there has been much interest in exploring the effect of costly punishment on human cooperation[8–23]. Costly punishment means paying a cost for another individual to incur a cost. It has been suggested that costly punishment promotes cooperation even in non-repeated games and without any possibility of reputation effects[10]. But most of our interactions are repeated and reputation is always at stake. Thus, if costly punishment is important in promoting cooperation, it must do so in a repeated setting. We have performed experiments in which, in each round of a repeated game, people choose between cooperation, defection and costly punishment. In control experiments, people could only cooperate or defect. Here we show that the option of costly punishment increases the amount of cooperation but not the average payoff of the group. Furthermore, there is a strong negative correlation between total payoff and use of costly punishment. Those people who gain the highest total payoff tend not to use costly punishment: winners don't punish. This suggests that costly punishment behaviour is maladaptive in cooperation games and might have evolved for other reasons.

The essence of cooperation is described by the Prisoner's Dilemma. Two players have a choice between cooperation, C, and defection, D. If both players cooperate they get more than if both defect, but defecting against a cooperator leads to the highest payoff, while cooperating with a defector leads to the lowest payoff. One way to construct a Prisoner's Dilemma is by assuming that cooperation implies paying a cost for the other person to receive a benefit, whereas defection implies taking something away from the other person (Fig. 1).

Without any mechanism for the evolution of cooperation, natural selection favours defection. However, several such mechanisms have been proposed, including direct and indirect reciprocity[7]. Direct reciprocity means there are repeated encounters between the same two individuals, and my behaviour depends on what you have done to me[1–6]. Indirect reciprocity means there are repeated encounters within a group; my behaviour also depends on what you have done to others.

Costly (or altruistic) punishment, P, means that one person pays a cost for another person to incur a cost. People are willing to use costly punishment against others who have defected[8–18]. Costly punishment is not a mechanism for the evolution of cooperation[7] but requires a mechanism for its evolution[19–23]. Like the idea of reputation effects[24], costly punishment is a form of direct or indirect reciprocity: if I punish you because you have defected against me, direct reciprocity is used; if I punish you because you have defected with others, indirect reciprocity is at work. The concept of costly punishment suggests that the basic game should be extended from two possible behaviours (C and D) to three (C, D and P). Here we investigate the consequences of this extension for the repeated Prisoner's Dilemma.

A total of 104 subjects participated in repeated Prisoner's Dilemma experiments at the Harvard Business School Computer Lab for Experimental Research. Participants interacted anonymously in pairwise encounters by means of computer screens. Subjects did not know how long each interaction would last, but knew that the probability of another round was 0.75 (as in ref. 25). In any given round, the subjects chose simultaneously between all available options, which were presented in a neutral language. After each round, the subjects were shown the other person's choice as well as both payoff scores. At the end of the interaction, the participants were presented with the final scores and then randomly rematched for another interaction.

We performed two control experiments (C1 and C2) and two treatments (T1 and T2). In the control experiments, people played a standard repeated Prisoner's Dilemma. In each round they could either cooperate or defect. Cooperation meant paying 1 unit for the other person to receive 2 units (in C1 and T1) or 3 units (in C2 and T2). Defection meant gaining 1 unit at a cost of 1 for the other person. In the treatments, people had three options in every round: cooperate, defect or punish. Punishment meant paying 1 unit for the other person to lose 4. We used a 4:1 punishment technology because it has been shown to be more effective in promoting cooperation than

a

Your move	You get	Other gets
C	$-c$	$+b$
D	$+d$	$-d$
P	$-\alpha$	$-\beta$

b

	C	D	P
C	$b-c$	$-d-c$	$-\beta-c$
D	$b+d$	0	$-\beta+d$
P	$b-\alpha$	$-d-\alpha$	$-\beta-\alpha$

c

	C	D	P
C	1	−2	−5
D	2	0	−3
P	1	−2	−5

d

	C	D	P
C	2	−2	−5
D	4	0	−3
P	2	−2	−5

Figure 1 | Payoff values. a, The game is formulated in terms of unilateral moves. There is the choice between cooperation (C), defection (D) and costly punishment (P). Cooperation means paying a cost c for the other person to get a benefit b. Defection means earning a payoff d at a cost d to the other person. Punishment means paying a cost α for the other person to incur a cost β. **b**, The payoff matrix is constructed from these unilateral moves. **c**, **d**, The actual payoff matrices of our experiments: C1 and T1 (**c**); C2 and T2 (**d**).

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other ratios[13]. The resulting payoff matrices are shown in Fig. 1. See Supplementary Information for more details.

Figure 2 shows some examples of games that occurred in the treatments T1 and T2. A number of games were all-out cooperation. Sometimes cooperation could be maintained by forgiving an opponent's defection. At other times, defection in response to defection was able to restore cooperation. Typically, costly punishment did not re-establish cooperation. In some cases, costly punishment provoked counter-punishment, thereby assuring mutual destruction. Giving people the option of costly punishment can also lead to unprovoked first strikes, with disastrous consequences.

Comparing the two control experiments, C1 and C2, we find that the frequency of cooperation increases as the benefit-to-cost ratio increases. In C1, 21.2% of decisions are cooperation, in contrast with 43.0% in C2. For both parameter choices, cooperation is a sub-game perfect equilibrium. Comparing each control experiment with its corresponding treatment, we find that punishment increases the frequency of cooperation. In T1 and T2, 52.4% and 59.7% of all decisions are cooperation.

Punishment, however, does not increase the average payoff. In T1 and T2, we observe that 7.6% and 5.8% of decisions are punishment, P. We find no significant difference in the average payoff when comparing C1 with T1 and C2 with T2. Punishment therefore has no benefit for the group, which makes it hard to argue that punishment might have evolved by group selection[22].

Examining the data of experiments T1 and T2 at the individual level, we find no correlation between the use of cooperation or defection and payoff, but a strong negative correlation between the use of punishment and payoff (Fig. 3). In experiment T1, the five top-ranked players, who earned the highest total payoff, have never used costly punishment. In both experiments, the players who end up with the lowest payoff tend to punish most often. Hence, for maximizing the overall income it is best never to punish: winners don't punish (Fig. 3).

It might be that the winners of our experiment were merely lucky in that they were paired with people against whom punishment was not necessary. To test this hypothesis, we analysed the correlation between payoff and the first-order conditional strategies used by people. Figure 4 illustrates a strong negative correlation between payoff and the probability to use punishment, P, after the opponent has defected, D. Winners tend to respond by using D against D, whereas losers use P against D. The response to another person's defection is the only strategic feature that is clearly correlated with winning or losing the game. Winners play a tit-for-tat-like strategy[2,4], whereas losers use costly punishment.

It could be that using costly punishment becomes more beneficial as the game progresses. To test this possibility, we separately analysed the data from the last one-quarter of all interactions. Again, it remains true that there is a strong negative correlation between an individual's payoff and that individual's use of costly punishment.

In previous experiments, punishment was usually offered as a separate option after one or several rounds of a public goods game. The public goods game is a multi-person Prisoner's Dilemma, in which each player can invest a certain sum into a common pool,

	Decisions	Payoff in this interaction	Final rank
a	Nice people finish first		
	C C C C	8	1
	C C C C	8	2
b	Punish and perish		
	C P P P P	−10	25
	D D D D D	−9	22
c	Defection restores cooperation		
	C D D C D C	10	15
	D D C C C C	4	9
d	Turning the other cheek		
	C C C C C	2	6
	D D C C C	14	19
e	Mutually assured destruction		
	C P P P D D	−20	30
	D D P P P P	−14	25
f	Revenge is not so sweet		
	C C C P D D P P P	−6	24
	C C D D D D D D D	−4	22
g	A 'pre-emptive strike'		
	C P D	2	29
	C C D	−4	24

Figure 2 | Games people played. There were 1,230 pairwise repeated interactions, each lasting between one and nine rounds. Some examples are given (**b**, **e** and **g** are from T1; the others are from T2). The two players' moves, the cumulative payoff of that interaction and the final rank of each player (sorted from highest to lowest payoff) are shown. **a**, All-out cooperation between two top-ranked players. **b**, Punish and perish. **c**, Defection for defection can sometimes restore cooperation. **d**, Turning the other cheek can also restore cooperation. **e**, Mutual punishment is mutual destruction. **f**, Punishment does not restore cooperation. Player 1 punishes a defection, which leads to mutual defection. Then player 1 is unsatisfied and deals out more punishment. **g**, "Guns don't kill people, people kill people." (Punishment itself is not destructive, only the people who use it.) Here, an unprovoked first strike destroys cooperation. The option to punish allows irrational people to inflict harm on the undeserving.

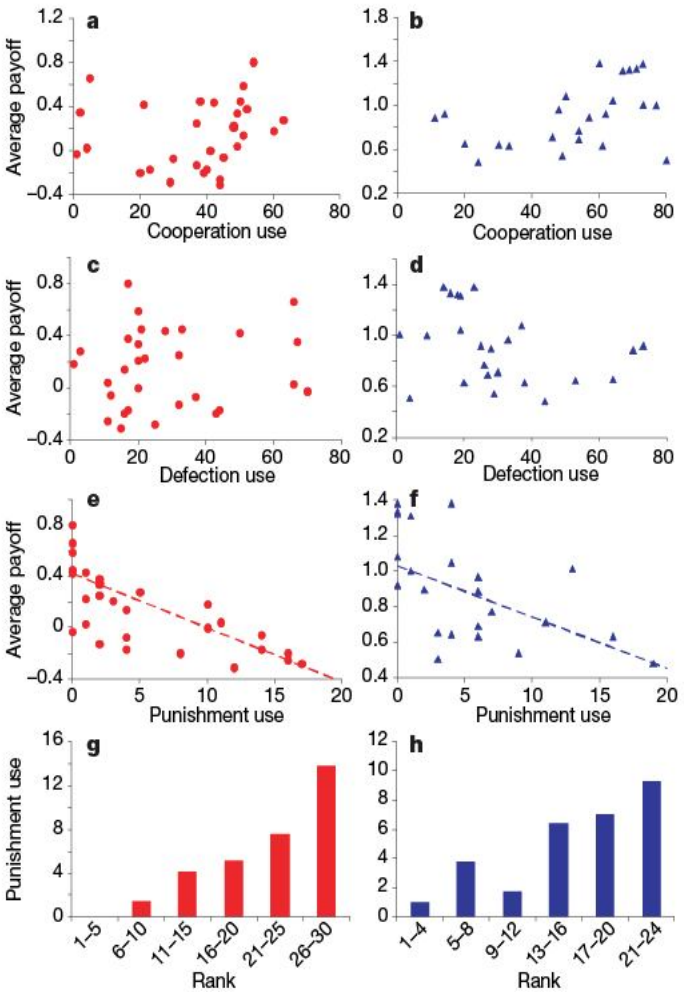

Figure 3 | Punish and perish. In both treatments T1 (red; $b/c = 2$) and T2 (blue; $b/c = 3$), there is no correlation between average payoff per round and use of cooperation (quantile regression; **a**, $P = 0.33$; **b**, $P = 0.21$) or between average payoff per round and use of defection (**c**, $P = 0.66$; **d**, $P = 0.36$). However, there is a significant negative correlation between average payoff per round and punishment use in both treatments (**e**, slope $= -0.042$, $P < 0.001$; **f**, slope $= -0.029$, $P = 0.015$). Punishment use is the overriding determinant of payoff. The x axis in **a**–**f** shows the total number of moves of the given type made over the course of the experiment. **g**, **h**, Ranking players according to their total payoff shows a clear trend: players with lower rank (higher payoffs) punish less than players with higher rank (lower payoff).

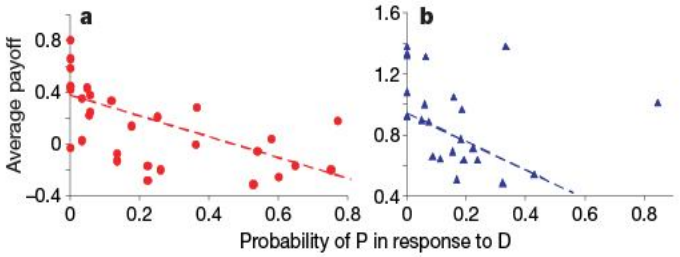

Figure 4 | Tit-for-tat prevails over costly punishment. Lower payoffs are correlated not only with punishment use, but also specifically with choosing to punish after the opponent has defected. The probability of punishing immediately after a co-player's defection is negatively correlated with the average payoff per round, both in T1 (**a**; $b/c = 2$) and in T2 (**b**; $b/c = 3$) (quantile regression; **a**, slope $= -0.81$, $P < 0.001$; **b**, slope $= -0.94$, $P = 0.015$). Thus, the lower payoffs of punishers were not caused by the bad luck of interacting with defectors. Winners use a tit-for-tat-like approach (D for D), whereas losers use costly punishment (P for D).

which is then multiplied by a factor and equally divided between all players irrespective of whether they have invested or not[26]. After the public goods game, people are asked if they want to pay money for others to lose money. People are willing to use this option to punish those who have invested nothing or only very little, and the presence of this option has been found to increase contributions[8,10].

Careful analysis, however, has revealed that, in most cases, punishment does not increase the average payoff. In some experiments, punishment reduces the average payoff[9,10,12,27], whereas in others it does not lead to a significant change[11,14,15]. Only once has punishment been found to increase the average payoff[13]. The higher frequency of cooperation is usually offset by the cost of punishment, which affects both the punisher and the punished. Our findings are in agreement with this observation: the option of costly punishment does not increase the average payoff of the group. It is possible, however, that in longer experiments and for particular parameter values punishment might increase the average payoff.

It is sometimes argued that costly punishment is a mechanism for stabilizing cooperation in anonymous, one-shot games. But whether or not this is the case seems to be of little importance, because most of our interactions occur in a context where repetition is possible and reputation matters. For millions of years of human evolution, our ancestors have lived in relatively small groups in which people knew each other. Interactions in such groups are certainly repeated and open ended. Thus, our strategic instincts have been evolving in situations where it is likely that others either directly observe my actions or eventually find out about them. In addition, in modern life most of our interactions occur with people whom we meet frequently. Typically, we can never rule out 'subsequent rounds'. Therefore, if costly punishment is important for the evolution of human cooperation, then it must have a beneficial role in the setting of repeated games. Our findings do not support this claim.

We also believe that our current design has some additional advantages over previous ones. In our setting, costly punishment is always one of three options. Hence, there is an opportunity cost for using punishment, because the subject forfeits the opportunity to cooperate or to defect. Our design also minimizes the experimenter and participant demand effects[28], because there are always several options[27]. In many previous experiments retaliation for punishment is not possible[9–16,27], but it is a natural feature of our setting.

Thus, our data show that costly punishment strongly disfavours the individual who uses it and hence it is opposed by individual selection in cooperation games in which direct reciprocity is possible. We conclude that costly punishment might have evolved for reasons other than promoting cooperation, such as coercing individuals into submission and establishing dominance hierarchies[20,29]. Punishment might enable a group to exert control over individual behaviour. A stronger individual could use punishment to dominate weaker ones. People engage in conflicts and know that conflicts can carry costs. Costly punishment serves to escalate conflicts, not to moderate them. Costly punishment might force people to submit, but not to cooperate. It could be that costly punishment is beneficial in these other games, but the use of costly punishment in games of cooperation seems to be maladaptive. We have shown that in the framework of direct reciprocity, winners do not use costly punishment, whereas losers punish and perish.

METHODS SUMMARY

A total of 104 subjects (45 women, 59 men, mean age 22.2 years) from Boston-area colleges and universities participated voluntarily in a modified repeated Prisoner's Dilemma game at the Harvard Business School Computer Lab for Experimental Research (CLER). The lab consists of 36 computers, which are visually partitioned. The participants interacted anonymously through the software z-Tree[30] and were from a number of different schools and a wide range of fields of study; it was therefore unlikely that any subject would know more than one other person in the room. We asked subjects for their sex and major field of study. No significant difference in level of cooperation, punishment use or payoff was found between males and females, or between economics majors and non-economics majors (Mann–Whitney test, $P > 0.05$ for all sessions). Subjects were not allowed to participate in more than one session of the experiment. In all, four sessions were conducted in April and May 2007, with an average of 26 participants playing an average of 24 interactions, for an average of 79 total rounds per subject.

Each experiment was begun by reading instructions (included in the Supplementary Information), answering two test questions to verify understanding of the payoffs, and playing a practice interaction against another subject. At the start of each new interaction, subjects were unaware of the previous decisions of the other player. After each round, the subjects were shown the other person's choice as well as both payoff scores. At the end of the interaction, the participants were presented with the final scores and then randomly rematched for another interaction.

In each session, the subjects were paid a $15 show-up fee. Each subject's final score summed over all interactions was multiplied by $0.10 to determine additional earned income. Thus, one game unit corresponded to $0.10. To allow for negative incomes while maintaining the $15 show-up fee, $5 was added to each subject's earned income at the end of the session. Subjects were informed of this extra $5 at the beginning of the session. The average payment per subject was $26 and the average session length was 1.25 h.

Received 13 November 2007; accepted 15 January 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements Support from the John Templeton Foundation, the National Science Foundation (NSF)–National Institutes of Health joint program in mathematical biology, the Jan Wallander Foundation (A.D.) and an NSF grant (D.F.) is gratefully acknowledged. The Program for Evolutionary Dynamics is sponsored by J. Epstein.

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LETTERS

Identifying natural images from human brain activity

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A challenging goal in neuroscience is to be able to read out, or decode, mental content from brain activity. Recent functional magnetic resonance imaging (fMRI) studies have decoded orientation[1,2], position[3] and object category[4,5] from activity in visual cortex. However, these studies typically used relatively simple stimuli (for example, gratings) or images drawn from fixed categories (for example, faces, houses), and decoding was based on previous measurements of brain activity evoked by those same stimuli or categories. To overcome these limitations, here we develop a decoding method based on quantitative receptive-field models that characterize the relationship between visual stimuli and fMRI activity in early visual areas. These models describe the tuning of individual voxels for space, orientation and spatial frequency, and are estimated directly from responses evoked by natural images. We show that these receptive-field models make it possible to identify, from a large set of completely novel natural images, which specific image was seen by an observer. Identification is not a mere consequence of the retinotopic organization of visual areas; simpler receptive-field models that describe only spatial tuning yield much poorer identification performance. Our results suggest that it may soon be possible to reconstruct a picture of a person's visual experience from measurements of brain activity alone.

Imagine a general brain-reading device that could reconstruct a picture of a person's visual experience at any moment in time[6]. This general visual decoder would have great scientific and practical use. For example, we could use the decoder to investigate differences in perception across people, to study covert mental processes such as attention, and perhaps even to access the visual content of purely mental phenomena such as dreams and imagery. The decoder would also serve as a useful benchmark of our understanding of how the brain represents sensory information.

How do we build a general visual decoder? We consider as a first step the problem of image identification[3,7,8]. This problem is analogous to the classic 'pick a card, any card' magic trick. We begin with a large, arbitrary set of images. The observer picks an image from the set and views it while brain activity is measured. Is it possible to use the measured brain activity to identify which specific image was seen?

To ensure that a solution to the image identification problem will be applicable to general visual decoding, we introduce two challenging requirements[6]. First, it must be possible to identify novel images. Conventional classification-based decoding methods can be used to identify images if brain activity evoked by those images has been measured previously, but they cannot be used to identify novel images (see Supplementary Discussion). Second, it must be possible

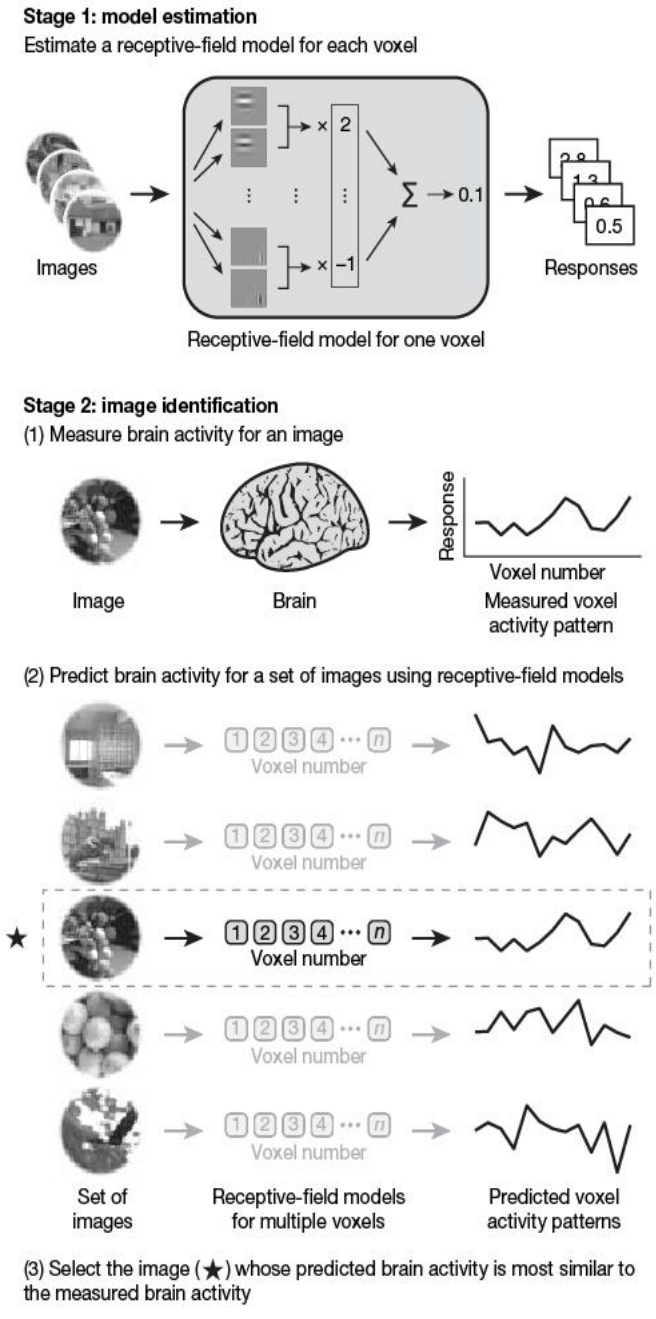

Figure 1 | Schematic of experiment. The experiment consisted of two stages. In the first stage, model estimation, fMRI data were recorded while each subject viewed a large collection of natural images. These data were used to estimate a quantitative receptive-field model[10] for each voxel. The model was based on a Gabor wavelet pyramid[11–13] and described tuning along the dimensions of space[3,14–19], orientation[1,2,20] and spatial frequency[21,22]. In the second stage, image identification, fMRI data were recorded while each subject viewed a collection of novel natural images. For each measurement of brain activity, we attempted to identify which specific image had been seen. This was accomplished by using the estimated receptive-field models to predict brain activity for a set of potential images and then selecting the image whose predicted activity most closely matches the measured activity.

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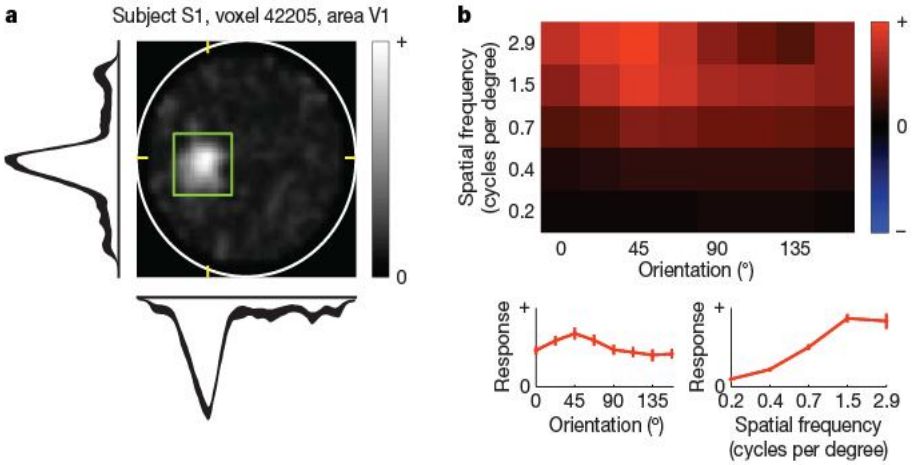

Figure 2 | Receptive-field model for a representative voxel. **a**, Spatial envelope. The intensity of each pixel indicates the sensitivity of the receptive field to that location. The white circle delineates the bounds of the stimulus ($20° \times 20°$) and the green square delineates the estimated receptive-field location. Horizontal and vertical slices through the spatial envelope are shown below and to the left. These intersect the peak of the spatial envelope, as indicated by yellow tick marks. The thickness of each slice profile indicates ± 1 s.e.m. This receptive field is located in the left hemifield, just below the horizontal meridian. **b**, Orientation and spatial frequency tuning curves. The top matrix depicts the joint orientation and spatial frequency tuning of the receptive field, and the bottom two plots give the marginal orientation and spatial frequency tuning curves. Error bars indicate ± 1 s.e.m. This receptive field has broadband orientation tuning and high-pass spatial frequency tuning. For additional receptive-field examples and population summaries of receptive-field properties, see Supplementary Figs 9–11.

to identify natural images. Natural images have complex statistical structure[9] and are much more difficult to parameterize than simple artificial stimuli such as gratings or pre-segmented objects. Because neural processing of visual stimuli is nonlinear, a decoder that can identify simple stimuli may fail when confronted with complex natural images.

Our experiment consisted of two stages (Fig. 1). In the first stage, model estimation, fMRI data were recorded from visual areas V1, V2 and V3 while each subject viewed 1,750 natural images. We used these data to estimate a quantitative receptive-field model[10] for each voxel (Fig. 2). The model was based on a Gabor wavelet pyramid[11–13] and described tuning along the dimensions of space[3,14–19], orientation[1,2,20] and spatial frequency[21,22]. (See Supplementary Discussion for a comparison of our receptive-field analysis with those of previous studies.)

In the second stage, image identification, fMRI data were recorded while each subject viewed 120 novel natural images. This yielded 120 distinct voxel activity patterns for each subject. For each voxel activity pattern we attempted to identify which image had been seen. To do this, the receptive-field models estimated in the first stage of the experiment were used to predict the voxel activity pattern that would be evoked by each of the 120 images. The image whose predicted voxel activity pattern was most correlated (Pearson's r) with the measured voxel activity pattern was selected.

Identification performance for one subject is illustrated in Fig. 3. For this subject, 92% (110/120) of the images were identified correctly (subject S1), whereas chance performance is just 0.8% (1/120). For a second subject, 72% (86/120) of the images were identified correctly (subject S2). These high performance levels demonstrate the validity of our decoding approach, and indicate that our receptive-field models accurately characterize the selectivity of individual voxels to natural images.

A general visual decoder would be especially useful if it could operate on brain activity evoked by a single perceptual event. However, because fMRI data are noisy, the results reported above were obtained using voxel activity patterns averaged across 13 repeated trials. We therefore attempted identification using voxel activity patterns from single trials. Single-trial performance was 51% (834/1620) and 32% (516/1620) for subjects S1 and S2, respectively (Fig. 4a); once again, chance performance is just 0.8% (13.5/1620). These results suggest that it may be feasible to decode the content of perceptual experiences in real time[7,23].

We have so far demonstrated identification of a single image drawn from a set of 120 images, but a general visual decoder should be able to handle much larger sets of images. To investigate this issue, we measured identification performance for various set sizes up to 1,000 images (Fig. 4b). As set size increased tenfold from 100 to 1,000, performance only declined slightly, from 92% to 82% (subject S1,

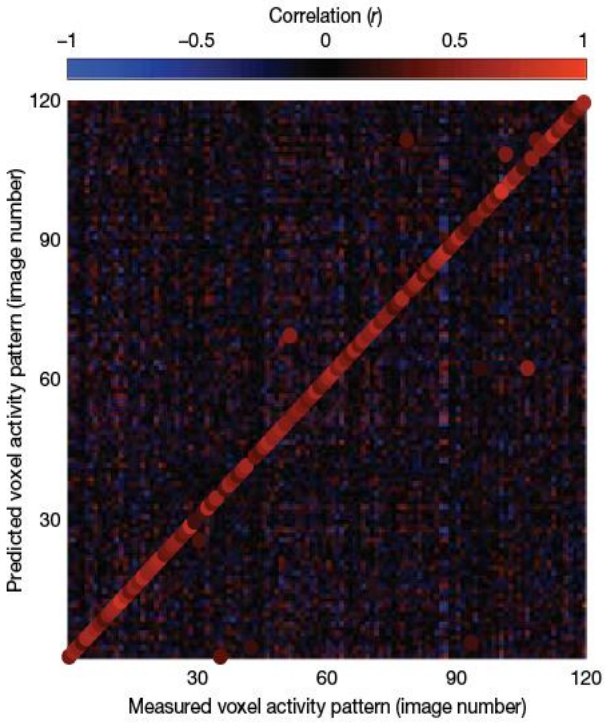

Figure 3 | Identification performance. In the image identification stage of the experiment, fMRI data were recorded while each subject viewed 120 novel natural images that had not been used to estimate the receptive-field models. For each of the 120 measured voxel activity patterns, we attempted to identify which image had been seen. This figure illustrates identification performance for one subject (S1). The colour at the mth column and nth row represents the correlation between the measured voxel activity pattern for the mth image and the predicted voxel activity pattern for the nth image. The highest correlation in each column is designated by an enlarged dot of the appropriate colour, and indicates the image selected by the identification algorithm. For this subject 92% (110/120) of the images were identified correctly.

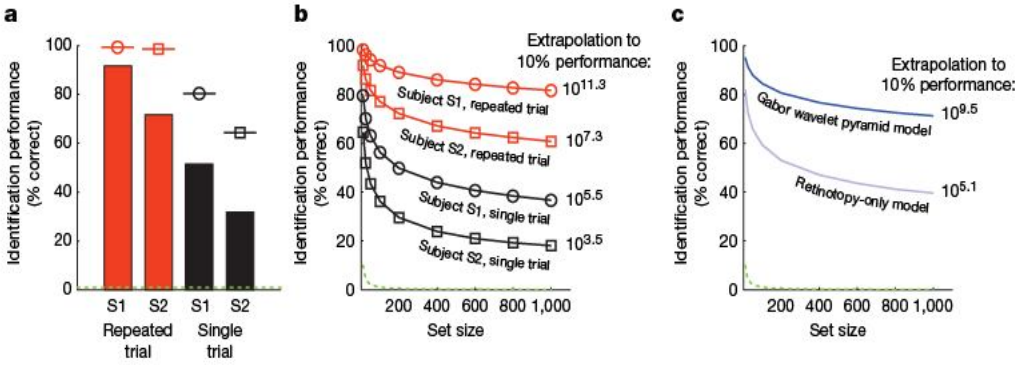

Figure 4 | Factors that impact identification performance. **a**, Summary of identification performance. The bars indicate empirical performance for a set size of 120 images, the marker above each bar indicates the estimated noise ceiling (that is, the theoretical maximum performance given the level of noise in the data), and the dashed green line indicates chance performance. The noise ceiling estimates suggest that the difference in performance across subjects is due to intrinsic differences in the level of noise. **b**, Scaling of identification performance with set size. The *x* axis indicates set size, the *y* axis indicates identification performance, and the number to the right of each line gives the estimated set size at which performance declines to 10% correct. In all cases performance scaled very well with set size. **c**, Retinotopy-only model versus Gabor wavelet pyramid model. Identification was attempted using an alternative retinotopy-only model that captures only the location and size of each voxel's receptive field. This model performed substantially worse than the Gabor wavelet pyramid model, indicating that spatial tuning alone is insufficient to achieve optimal identification performance. (Results reflect repeated-trial performance averaged across subjects; see Supplementary Fig. 5 for detailed results.)

repeated trial). Extrapolation of these measurements (see Supplementary Methods) suggests that performance for this subject would remain above 10% even up to a set size of $10^{11.3}$ images. This is more than 100 times larger than the number of images currently indexed by Google ($10^{8.9}$ images; source: http://www.google.com/whatsnew/, 4 June 2007).

Early visual areas are organized retinotopically, and voxels are known to reflect this organization[14,16,18]. Could our results be a mere consequence of retinotopy? To answer this question, we attempted identification using an alternative model that captures the location and size of each voxel's receptive field but discards orientation and spatial frequency information (Fig. 4c). Performance for this retinotopy-only model declined to 10% correct at a set size of just $10^{5.1}$ images, whereas performance for the Gabor wavelet pyramid model did not decline to 10% correct until $10^{9.5}$ images were included in the set (repeated-trial performance extrapolated and averaged across subjects). This result indicates that spatial tuning alone does not yield optimal identification performance; identification improves substantially when orientation and spatial frequency tuning are included in the model.

To further investigate the impact of orientation and spatial frequency tuning, we measured identification performance after imposing constraints on the orientation and spatial frequency tuning of the Gabor wavelet pyramid model (Supplementary Fig. 8). The results indicate that both orientation and spatial frequency tuning contribute to identification performance, but that the latter makes the larger contribution. This is consistent with recent studies demonstrating that voxels have only slight orientation bias[1,2]. We also find that voxel-to-voxel variation in orientation and spatial frequency tuning contributes to identification performance. This reinforces the growing realization in the fMRI community that information may be present in fine-grained patterns of voxel activity[6].

To be practical our identification algorithm must perform well even when brain activity is measured long after estimation of the receptive-field models. To assess performance over time[2,4,6,23] we attempted identification for a set of 120 novel natural images that were seen approximately two months after the initial experiment. In this case 82% (99/120) of the images were identified correctly (chance performance 0.8%; subject S1, repeated trial). We also evaluated identification performance for a set of 12 novel natural images that were seen more than a year after the initial experiment. In this case 100% (12/12) of the images were identified correctly (chance performance 8%; subject S1, repeated trial). These results demonstrate that the stimulus-related information that can be decoded from voxel activity remains largely stable over time.

Why does identification sometimes fail? Inspection revealed that identification errors tended to occur when the selected image was visually similar to the correct image. This suggests that noise in measured voxel activity patterns causes the identification algorithm to confuse images that have similar features.

Functional MRI signals have modest spatial resolution and reflect haemodynamic activity that is only indirectly coupled to neural activity[24,25]. Despite these limitations, we have shown that fMRI signals can be used to achieve remarkable levels of identification performance. This indicates that fMRI signals contain a considerable amount of stimulus-related information[4] and that this information can be successfully decoded in practice.

Identification of novel natural images brings us close to achieving a general visual decoder. The final step will require devising a way to reconstruct the image seen by the observer, instead of selecting the image from a known set. Stanley and co-workers[26] reconstructed natural movies by modelling the luminance of individual image pixels as a linear function of single-unit activity in cat lateral geniculate nucleus. This approach assumes a linear relation between luminance and the activity of the recorded units, but this condition does not hold in fMRI[27,28].

An alternative approach to reconstruction is to incorporate receptive-field models into a statistical inference framework. In such a framework, receptive-field models are used to infer the most likely image given a measured activity pattern. This model-based approach has a long history in both theoretical and experimental neuroscience[29,30]. Recently, Thirion and co-workers[3] used it to reconstruct spatial maps of contrast from fMRI activity in human visual cortex. The success of the approach depends critically on how well the receptive-field models predict brain activity. The present study demonstrates that our receptive-field models have sufficient predictive power to enable identification of novel natural images, even for the case of extremely large sets of images. We are therefore optimistic that the model-based approach will make possible the reconstruction of natural images from human brain activity.

METHODS SUMMARY

The stimuli consisted of sequences of $20^\circ \times 20^\circ$ greyscale natural photographs (Supplementary Fig. 1a). Photographs were presented for 1 s with a delay of 3 s between successive photographs (Supplementary Fig. 1b). Subjects (S1: author T.N.; S2: author K.N.K.) viewed the photographs while fixating a central white square. MRI data were collected at the Brain Imaging Center at University of California, Berkeley using a 4 T INOVA MR scanner (Varian, Inc.) and a quadrature transmit/receive surface coil (Midwest RF, LLC). Functional BOLD data were recorded from occipital cortex at a spatial resolution of 2 mm × 2 mm × 2.5 mm and a temporal resolution of 1 Hz. Brain volumes were

reconstructed and then co-registered to correct differences in head positioning within and across scan sessions. The time-series data were pre-processed such that voxel-specific response time courses were deconvolved from the data. Voxels were assigned to visual areas based on retinotopic mapping data[17] collected in separate scan sessions.

In the model estimation stage of the experiment, a receptive-field model was estimated for each voxel. The model was based on a Gabor wavelet pyramid[11–13] (Supplementary Figs 2 and 3), and was able to characterize responses of voxels in early visual areas V1, V2 and V3 (Supplementary Table 1). Alternative receptive-field models were also used, including the retinotopy-only model and several constrained versions of the Gabor wavelet pyramid model. Details of these models and model estimation procedures are given in Supplementary Methods.

In the image identification stage of the experiment, the estimated receptive-field models were used to identify images viewed by the subjects, based on measured voxel activity. The identification algorithm is described in the main text. For details of voxel selection, performance for different set sizes, and noise ceiling estimation, see Supplementary Fig. 4 and Supplementary Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 16 June 2007; accepted 17 January 2008.
Published online 5 March 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work was supported by a National Defense Science and Engineering Graduate fellowship (K.N.K.), the National Institutes of Health, and University of California, Berkeley intramural funds. We thank B. Inglis for assistance with MRI, K. Hansen for assistance with retinotopic mapping, D. Woods and X. Kang for acquisition of whole-brain anatomical data, and A. Rokem for assistance with scanner operation. We also thank C. Baker, M. D'Esposito, R. Ivry, A. Landau, M. Merolle and F. Theunissen for comments on the manuscript. Finally, we thank S. Nishimoto, R. Redfern, K. Schreiber, B. Willmore and B. Yu for their help in various aspects of this research.

Author Contributions K.N.K. designed and conducted the experiment and was first author on the paper. K.N.K. and T.N. analysed the data. R.J.P. provided mathematical ideas and assistance. J.L.G. provided guidance on all aspects of the project. All authors discussed the results and commented on the manuscript.

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LETTERS

Memory CD4 T cells emerge from effector T-cell progenitors

Laurie E. Harrington[1]†, Karen M. Janowski[1], James R. Oliver[1], Allan J. Zajac[2] & Casey T. Weaver[1]

A hallmark of adaptive immunity is the generation of memory T cells that confer long-lived, antigen-specific protection against repeat challenges by pathogens[1–5]. Understanding the mechanisms by which memory T cells arise is important for rational vaccination strategies and improved therapeutic interventions for chronic infections and autoimmune disorders. The large clonal expansion of CD8 T cells in response to some infections has made the development of CD8 T-cell memory more amenable to study, giving rise to a model of memory cell differentiation in which a fraction of fully competent effector T cells transition into long-lived memory T cells[4,6,7]. Delineation of CD4 T-cell memory development has proved more difficult as a result of limitations on tracking the smaller populations of CD4 effector T cells generated during a pathogenic challenge[8–10], complicating efforts to determine whether CD4 memory T cells are direct descendants of effector T cells or whether they develop by alternative pathways[3,4]. Here, using two complementary cytokine reporter mouse models to identify interferon (IFN)-γ-positive effector T cells and track their fate, we show that the lineage relationship between effector and memory CD4 T cells resembles that for CD8 T cells responding to the same pathogen. We find that, in parallel with effector CD8 T cells, IFN-γ-positive effector CD4 T cells give rise to long-lived memory T cells capable of anamnestic responses to antigenic rechallenge.

Viral and intracellular bacterial infections stimulate type 1 (IFN-γ^+) CD4 and CD8 T-cell effector responses and induce the development of long-lived memory T cells[3,4]. To follow the fate of effector CD4 T cells and define possible links to memory, a bacterial artificial chromosome (BAC) transgenic mouse model, termed *Ifng/Thy1.1* BAC-In, was developed in which IFN-γ^+ effector cells could be identified by the expression of a Thy1.1 (CD90.1) reporter molecule[11]. After validating the fidelity of this model (Supplementary Fig. 1), transgenic and wild-type mice were infected with lymphocytic choriomeningitis virus (LCMV), and responding CD4 and CD8 T cells were analysed at the peak of the effector phase (day 8) for upregulation of the Thy1.1 reporter molecule (Fig. 1 and Supplementary Fig. 2)[7,8,12,13]. The magnitude of the CD8 effector response was substantially greater than that of the CD4 response (35% Thy1.1$^+$CD8 versus 12% Thy1.1$^+$CD4; Fig. 1a, and data not shown)[8–10,12,13]. CD4 and CD8 T cells marked by the Thy1.1 reporter molecule directly *ex vivo* displayed a surface phenotype associated with effector T cells; Thy1.1$^+$ CD4 and CD8 T cells were all CD44^{high} and predominantly CD62L^{low} and CD127^{low} (Fig. 1a)[14–18]. Further characterization of the CD8 T-cell response with the use of LCMV-specific major histocompatibility complex (MHC) class I tetramers indicated that similar frequencies of the D^b(GP33)$^+$ and D^b(NP396)$^+$ CD8 T cells expressed Thy1.1, and these cells were primarily CD62L^{low} and CD127^{low}, demonstrating no preferential expression of the transgenic reporter among effector subpopulations (Fig. 1b)[7,14,16,19–21]. Intracellular staining for IFN-γ after stimulation *ex vivo* with LCMV-derived MHC class II-restricted or MHC class I-restricted peptides showed that about one-third of LCMV-specific IFN-γ^+ CD4 and CD8 T cells expressed Thy1.1, and after stimulation with 12-*O*-tetradecanoylphorbol-13-acetate and ionomycin all of the Thy1.1$^+$ T cells were IFN-γ^+ (Fig. 1c, and data not shown)[8–10,13,22]. Similar findings were observed after infection with *Listeria monocytogenes* (Supplementary Fig. 3)[23,24]. Thus, the transgenic reporter faithfully identified a consistent fraction of LCMV-specific and *Listeria*-specific effector CD4 and CD8 T cells generated *in vivo* in response to infection.

To investigate the fate of IFN-γ^+ effector T cells after the resolution of LCMV infection, the persistence and phenotype of T cells were examined during the memory phase (more than 40 days after infection). Both Thy1.1$^+$ CD4 and CD8 T cells were observed directly *ex vivo* from LCMV-immune *Ifng/Thy1.1* BAC-In mice, with the expected reductions in cell frequencies compared with those on day 8 (about 4% and 19%, respectively; Supplementary Fig. 2a). Thy1.1$^+$ T cells showed signatures of effector-memory T cells (CD62L^{low} and CD127^{high})[7,14,16,17,19,25,26] and retained the ability to produce IFN-γ in response to stimulation with LCMV-specific peptides (Supplementary Fig. 2b, and data not shown). These data imply that memory development for CD4 T cells resembles that of CD8 T cells, because a proportion of IFN-γ^+ effector T cells of both lineages survived into the memory phase and expressed surface markers characteristic of effector-memory cells.

Although these data suggested that memory CD4 T cells differentiate from effector T-cell progenitors, they did not exclude the possibility that the memory cells develop by a distinct pathway associated with the upregulation of the Thy1.1 reporter molecule after the effector phase[3–6,27–29]. To address this, IFN-γ^+ (Thy1.1$^+$) effector CD4 T cells were isolated from LCMV-infected *Ifng/Thy1.1* BAC-In mice at the peak of the effector response and transferred into CD45.1 congenic hosts at the same stage of infection (Fig. 2). A complement of IFN-γ^+ effector CD8 T cells from donor mice was also transferred to simultaneously track the fate of LCMV-specific effector CD8 T cells during the generation of immunological memory. After viral clearance and transition to the memory phase of the response, both CD4 and CD8 T cells of donor origin were readily detected in recipient mice that received IFN-γ^+ (Thy1.1$^+$) effector T cells (Fig. 2, and data not shown). The antigenic specificity of surviving donor-derived CD4 and CD8 T cells was confirmed by stimulation *ex vivo* with LCMV-derived peptides; a substantial fraction of the donor CD4 and CD8 T cells produced IFN-γ (about 8.5% and about 25%, respectively; Fig. 2b). This represented a marked enrichment of LCMV-specific T cells within the donor cell pool compared with the endogenous, recipient (CD45.1) LCMV-specific T-cell responses, and established the persistence of both CD4 and CD8 IFN-γ^+ effectors.

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Examination of cell surface marker expression on donor CD4 and CD8 T cells revealed that most donor T cells displayed a memory phenotype; the donor-derived CD4 and CD8 T cells maintained expression of Thy1.1, upregulated CD11a, CD44, CD122 and CD127, and displayed low levels of CD43 (Fig. 2c and Supplementary Fig. 4)[14,15,17–19]. An interesting difference observed was that a greater proportion of donor-derived CD4 T cells remained $CD62L^{low}$ in comparison with the transferred CD8 T cells. Both CD4 and CD8 donor-derived T cells showed enhanced homeostatic proliferation compared with non-memory endogenous T cells ($CD45.1^+CD44^{low}$), which was comparable to that of the endogenous pool of memory T cells ($CD45.1^+CD44^{high}$) (Fig. 2d). In addition, the LCMV-specific CD4 T cells that persisted produced both IL-2 and IFN-γ on re-stimulation (Supplementary Fig. 5). These data are consistent with the findings from intact LCMV-immune *Ifng/Thy1.1* BAC-In mice and establish that IFN-γ^+ effector CD4 T cells represent progenitors from which long-lived effector-memory cells develop, paralleling CD8 memory development in response to the same pathogenic challenge[6,7,16,19,25,26].

A fundamental feature of memory T cells is enhanced responsiveness to secondary challenge with antigen[2–5]. To compare CD4 and CD8 recall responses, recipient mice that had received simultaneous transfers of IFN-γ^+ ($Thy1.1^+$) effector CD4 and CD8 T cells at the peak of the effector response were challenged again with LCMV. All mice in these analyses were immune to LCMV, permitting a direct comparison of recall responses of donor ($CD45.2^+$) and endogenous ($CD45.1^+$) memory T cells. As shown in Fig. 3a, expansion of both donor-derived CD4 and CD8 T cells was detectable five days after the repeated LCMV challenge. In comparison with levels before the challenge, frequencies of donor CD4 T cells increased substantially after the challenge, with significantly enhanced frequencies of donor CD4 T cells (about 30%) that produced IFN-γ after stimulation with LCMV-specific epitopes (Fig. 3b). Donor CD8 T cells showed a similar recall response, with more than 45% of the $CD45.2^+$ CD8 T cells producing IFN-γ in response to the LCMV-specific peptides examined. Thus, the IFN-γ^+ effector CD4 T cells, like IFN-γ^+ effector CD8 T cells, were not only capable of persisting on adoptive transfer but also mounted a strong anamnestic response, a cardinal property of memory.

Although the foregoing studies established the transition of IFN-γ^+ effector CD4 T cells into the memory compartment, they did not address the fate of antigen-stimulated IFN-γ^- CD4 T cells after activation. Because the *Ifng/Thy1.1* BAC-In model does not mark all IFN-γ^+ cells, a second IFN-γ reporter mouse model was developed. Mice in which the Thy1.1 reporter was introduced into

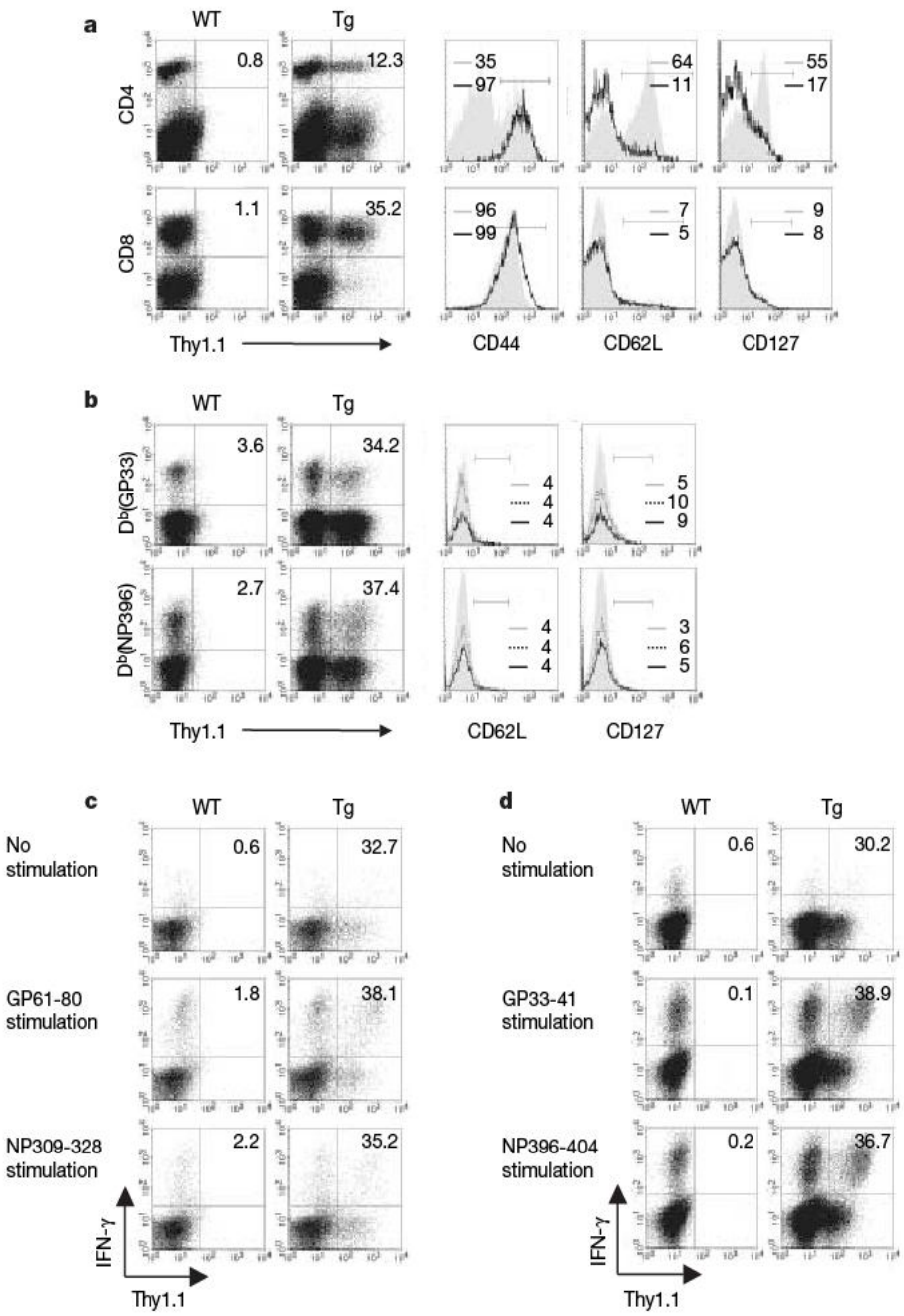

Figure 1 | Tracking IFN-γ^+ effector CD4 and CD8 T cells in *Ifng/Thy1.1* BAC-In Tg mice. Splenocytes from wild-type (WT) or *Ifng/Thy1.1* BAC-In (Tg) mice were analysed 8 days post LCMV infection. **a**, Left: plots showing frequencies of CD4 or CD8 T cells positive for Thy1.1 expression directly *ex vivo*. Right: histograms illustrating surface marker expression on WT (shaded area) or $Thy1.1^+$ (black line) CD4 or CD8 T cells from WT or Tg mice, respectively. The percentage of cells within each gate (defined by the horizontal H-shaped bars) is indicated. **b**, Left: frequencies of $Thy1.1^+$ D^b(GP33) and D^b(NP396) $tetramer^+$ CD8 T cells (plots gated on CD8 T cells). Right: CD62 ligand (CD62L) and CD127 levels determined on WT (shaded area), Tg $Thy1.1^-$ (dotted line) and Tg $Thy1.1^+$ (solid line) $tetramer^+$ effector CD8 T cells and the percentage of each cell population within the defined gates indicated. **c**, **d**, Thy1.1 expression by LCMV-specific IFN-γ^+ CD4 (**c**) and CD8 (**d**) T cells determined by intracellular cytokine staining after stimulation with the indicated peptide epitopes. Plots are gated on CD4 or CD8 T cells, and the frequencies of IFN-γ^+ cells that stained $Thy1.1^+$ are noted.

the endogenous *Ifng* genes were generated by gene targeting (*Ifng/Thy1.1* knock-in mouse) and were used to discriminate between IFN-γ^+ and IFN-γ^- cells (Supplementary Fig. 6). In the *Ifng/Thy1.1* knock-in model, and distinct from *Ifng/Thy1.1* BAC-In model in which the reporter transcript was stabilized by an exogenous 3′-UT element, reporter expression by T cells is transient and downregulated rapidly on effector T cells, reflecting the expression of Thy1.1 with IFN-γ from a bicistronic transcript containing endogenous 3′-UT elements (Fig. 4b and Supplementary Fig. 6, and data not shown). Accordingly, it is more difficult to time the isolation of reporter-positive and reporter-negative cells in an asynchronous *in vivo* clonal response (data not shown). For this reason, an *in vitro* approach was adopted to generate IFN-γ^+ and IFN-γ^- effector cells for assessment of cell fate (Fig. 4). Effector CD4 T cells were generated from *Ifng/Thy1.1* knock-in mice under T-helper type 1 (T_H1)-polarizing conditions and sorted into IFN-γ^- (Thy1.1$^-$) and IFN-γ^+ (Thy1.1$^+$) subsets followed by transfer into congenic CD45.1 mice (Fig. 4c, d). The IFN-γ^+ CD4 T cells showed modest expansion immediately after transfer compared with the IFN-γ^- CD4 T cells. However, both populations then showed a comparable early clonal contraction, followed by a more extended rate of decline that was not significantly different between groups (half-life of about 15 days; Fig. 4e, and data not shown). A significantly larger proportion of the persisting IFN-γ^+ CD4 T cells produced IFN-γ on re-stimulation, which is consistent with an enhanced recall effector response (Fig. 4f, g). Thus, both IFN-γ^+ and IFN-γ^- subpopulations from a type 1 CD4 effector pool give rise to memory cells, albeit with distinct potentials for a recall cytokine response.

Previous studies that have examined CD4 memory T-cell development have relied on transfers of heterogeneous populations of antigen-primed cells generated *ex vivo*, or have relied on surface markers that do not absolutely correlate with the effector status of individual cells, precluding definitive longitudinal studies of effector cell fate. Further, a recent study demonstrated that clonal populations of CD4 T cells are more efficiently activated and have substantially prolonged survival when activated at low, physiological precursor frequencies that limit intraclonal competition[30], indicating a need to track memory development in the context of pathogen-induced responses of the normal T-cell repertoire. The *Ifng/Thy1.1* BAC-In model, in which IFN-γ competent effectors derived from endogenous precursors can be stably marked *in vivo* after pathogenic challenge, has permitted the simultaneous tracking of pathogen-induced type 1 CD4 and CD8 effectors during memory transition from the normal precursor pool, and show that type 1 memory CD4 T cells, like

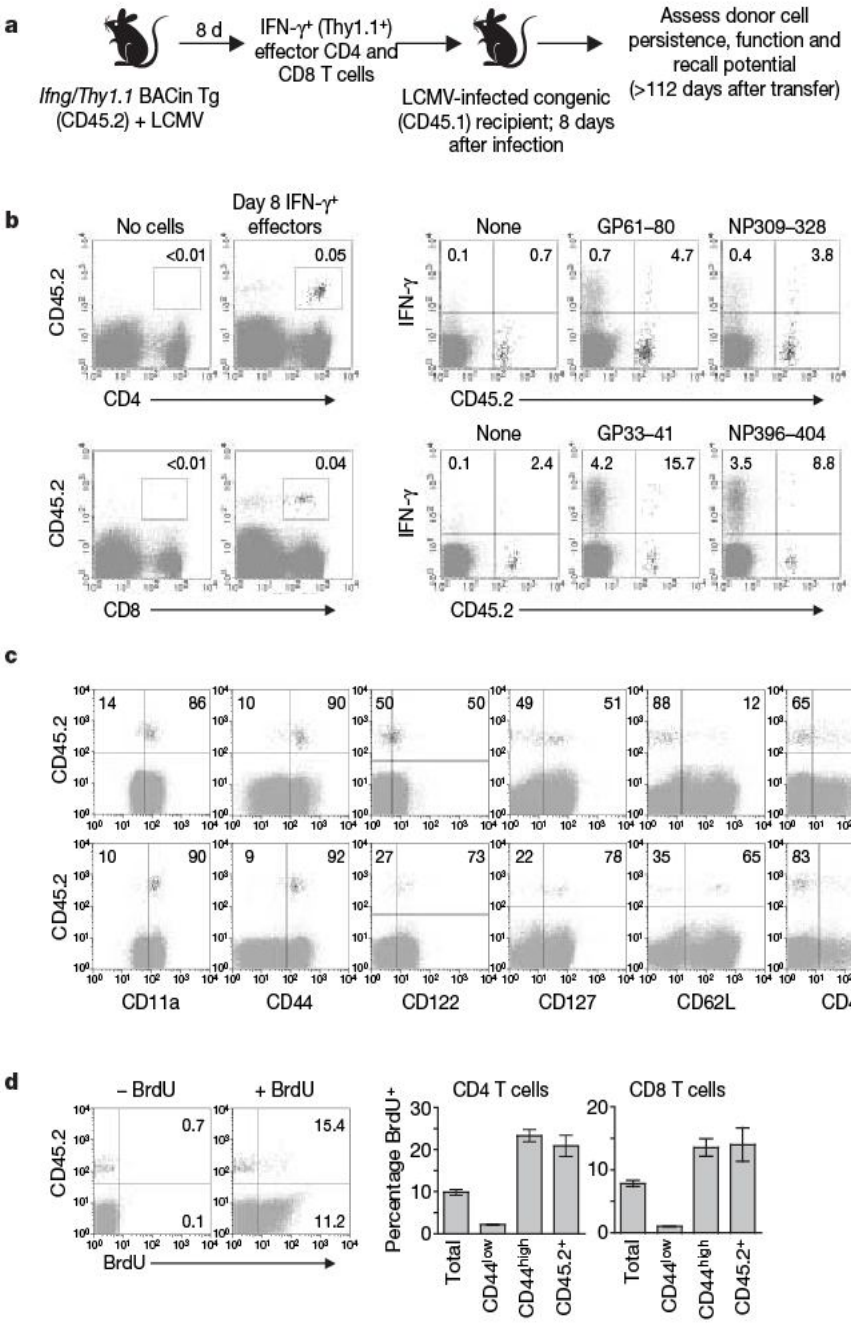

Figure 2 | Progeny of IFN-γ competent effector CD4 and CD8 T cells populate the memory T-cell compartment. **a**, Experimental design. **b–d**, Splenocytes of CD45.1 mice that received donor IFN-γ^+ (Thy1.1$^+$) effector T cells on day 8 after LCMV infection, or control mice that received no cell transfers, analysed more than 120 days after infection. **b**, Left: donor (CD45.2$^+$) CD4 and CD8 T cells were identified by flow cytometry and percentages of total CD4 and CD8 T cells that were CD45.2$^+$ are indicated. Right: intracellular IFN-γ staining was performed after stimulation with LCMV-derived peptide epitopes and percentages of donor (CD45.2$^+$) and host (CD45.2$^-$) CD4 T cells (top) or CD8 T cells (bottom) that produced IFN-γ were determined. **c**, Surface marker expression on gated CD4 (top row) and CD8 (bottom row) T cells is shown and the percentage of donor cells within each gate is indicated. **d**, Homeostatic proliferation of donor T cells was assessed by BrdU incorporation over an 8-day period. Mice that did not receive BrdU served as controls. Left: representative flow cytometry plots gated on CD4 T cells; the percentages of CD45.2$^+$ and CD45.2$^-$ CD4 T cells that stained with BrdU are indicated. Right: graphs indicating the percentages of specified CD4 and CD8 T-cell populations that stained with BrdU (results are means ± s.e.m.; $n = 3–5$ determinations).

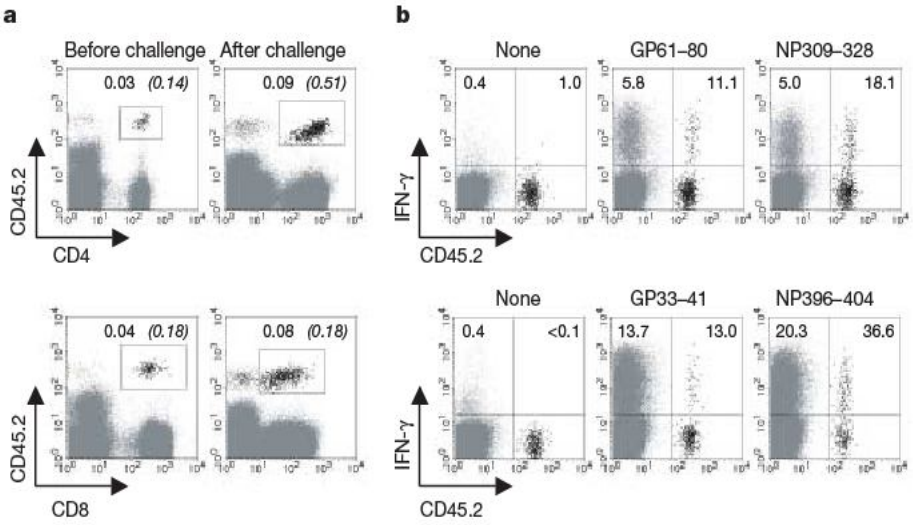

Figure 3 | Anamnestic responses conferred by memory CD4 T cells derived from IFN-γ$^+$ effector T cells. CD45.1 mice that received effector T-cell transfers as in Fig. 2 (82 days after LCMV infection; 74 days after transfer of IFN-γ$^+$ effector donor T cells) were challenged again with LCMV and analysed 5 days later. **a**, Donor (CD45.2$^+$) CD4 (top) and CD8 (bottom) T cells were quantified in the peripheral blood either before challenge (12 days after transfer) or after challenge (5 days after secondary LCMV infection). Values shown are the percentages of donor CD4 or CD8 T cells within the entire lymphocyte population (roman) and the percentages of total CD4 or CD8 T cells that were of donor origin (italics). **b**, The percentages of donor (CD45.2$^+$) or host (CD45.2$^-$) splenic T cells that produced IFN-γ after *ex vivo* stimulation with LCMV-specific peptides. Plots are gated on CD4 (top) or CD8 (bottom) T cells.

memory CD8 T cells, arise from IFN-γ$^+$ effectors, implying a common developmental programme used by both CD4 and CD8 memory T-cell subsets. Although further comparative studies to define better the relative long-term stability and recall function of memory CD4 T cells generated from IFN-γ$^+$ and IFN-γ$^-$ progeny will be needed, data from the complementary *Ifng/Thy1.1* knock-in

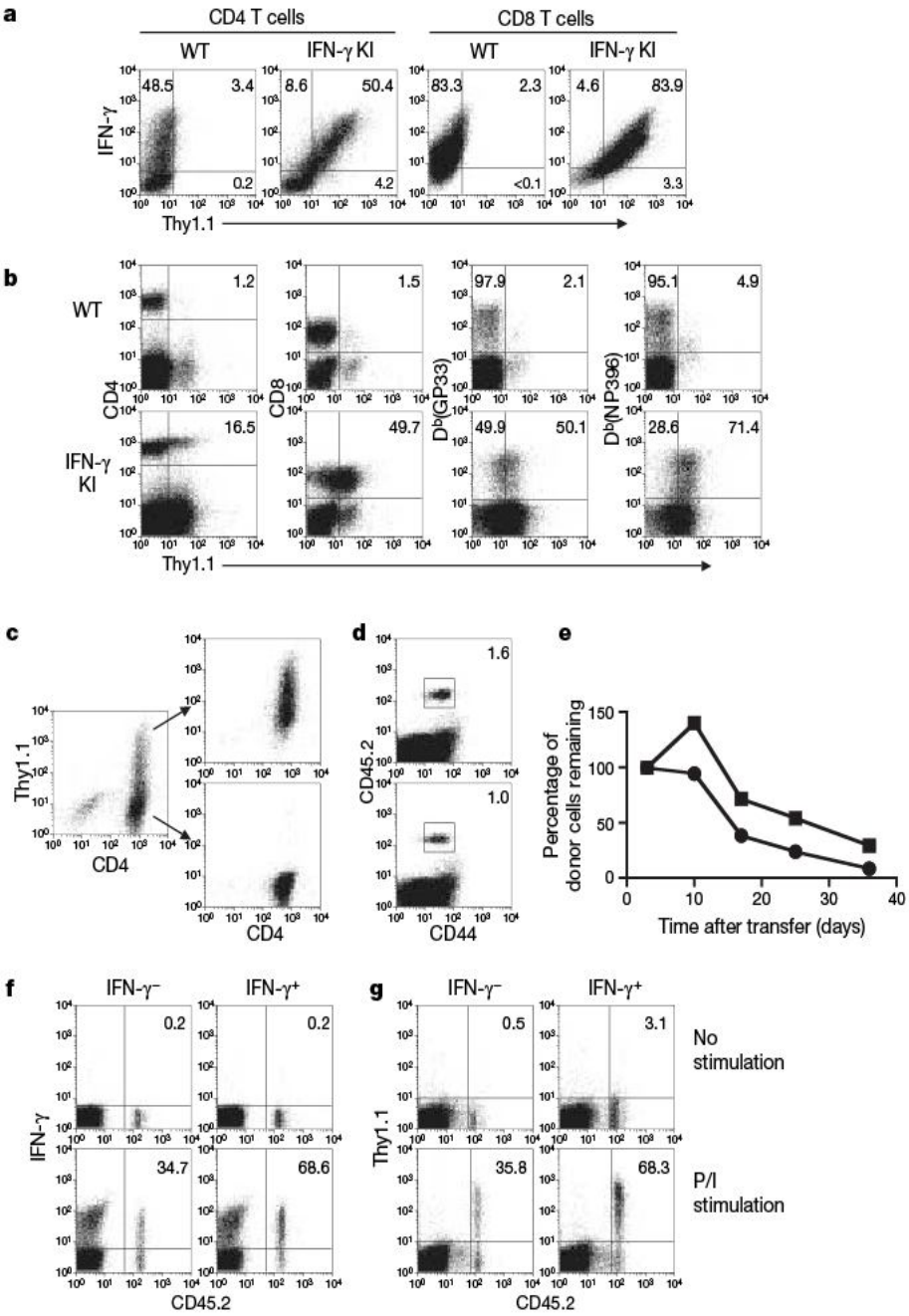

Figure 4 | Both IFN-γ$^+$ and IFN-γ$^-$ effector CD4 T cells persist *in vivo*. **a**, *In vitro* polarized CD4 and CD8 T cells from WT or *Ifng/Thy1.1* knock-in (IFN-γ KI) mice were stained intracellularly for IFN-γ and Thy1.1. The plots are gated on either CD4 or CD8 T cells and the percentage of cells in each quadrant is indicated. **b**, CD4 and CD8 T cells, or D^b(GP33) and D^b(NP396) tetramer$^+$ CD8 T cells from LCMV-infected WT or IFN-γ KI mice were analysed for Thy1.1 expression on day 8 after infection. Values indicate percentages of Thy1.1$^+$ cells within total CD4, total CD8 or tetramer$^+$ (CD8-gated) cell fractions. **c**, T_H1-polarized CD4 T cells from IFN-γ KI mice (CD45.2$^+$) were sorted into IFN-γ$^-$ (Thy1.1$^-$) and IFN-γ$^+$ (Thy1.1$^+$) populations before adoptive transfer into congenic (CD45.1$^+$) recipients. **d**, Mice that received IFN-γ$^+$ (top) or IFN-γ$^-$ (bottom) CD4 T-cell transfers were analysed for frequencies of donor (CD45.2$^+$) cells in the circulation 36 days after adoptive transfer. Values indicate percentages of total CD4 T cells of donor origin. **e**, Persistence of IFN-γ$^+$ (squares) or IFN-γ$^-$ (circles) effector CD4 T cells, tracked in the peripheral blood of recipient mice after transfer. Values are normalized to 100% on the basis of the frequency of donor cells on day 3 after transfer and are averages of two or three mice per group for each time point. **f**, **g**, The ability of persisting donor CD4 T cells to produce IFN-γ was determined by re-stimulation *ex vivo* and staining for intracellular IFN-γ (**f**) or Thy1.1 (**g**). Values indicate percentages of donor (CD45.2$^+$) cells that were IFN-γ$^+$ or Thy1.1$^+$ (CD4 T-cell gate).

model suggest similar survival characteristics but distinct functional potentials, suggesting that manoeuvres to enhance the potency of the effector pool in vaccination strategies will be beneficial, not deleterious, to long-lived protection. These findings provide a framework for deciphering the signals required for the generation and maintenance of memory CD4 T cells, which will be crucial for the development of rational strategies to manipulate both CD4 and CD8 memory T-cell compartments to enhance protective immunity or blunt autoimmunity.

METHODS SUMMARY

Generation of mice. The *Ifng/Thy1.1* BAC-In transgene was developed by replacing the first exon of the mouse *Ifng* gene on a bacterial artificial chromosome with the Thy1.1-coding sequence through recombineering, and the transgene was injected into single-cell C57BL/6 embryos by means of standard techniques. *Ifng/Thy1.1* knock-in mice were generated by gene targeting in C57BL/6 embryonic stem cells.

Mice and infections. C57BL/6J (wild-type) and B6.SJL-Ptprca Pep3b/BoyJ (CD45.1) mice were purchased from the Jackson Laboratory. Transgenic mouse strains were bred and maintained in accordance with IACUC guidelines. All LCMV and LM-OVA infections were performed with published strains and protocols.

Cell isolations. Thy1.1$^+$ CD4 and CD8 T cells were isolated from *Ifng/Thy1.1* BAC-In Tg mice by magnetic bead separation using Dynabeads (Dynal) in accordance with the manufacturers' directions. Thy1.1$^+$ and Thy1.1$^-$ *Ifng/Thy1.1* knock-in CD4 T cells were sorted by fluorescence-activated cell sorting before adoptive transfer.

***In vitro* activation of primary T-cells.** Purified CD4 and CD8 T cells were stimulated *in vitro* in the presence of irradiated feeder cells with anti-CD3 ($2.5\,\mu g\,ml^{-1}$) under type 1 polarizing conditions for the indicated periods.

Flow cytometry. Cell-surface and intracellular staining were performed with published protocols, and staining with bromodeoxyuridine (BrdU) was performed with the BrdU Flow Kit (BD Pharmingen) in accordance with the manufacturer's directions.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 6 November 2007; accepted 11 January 2008.
Published online 5 March 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank R. Hatton and C. Maynard, and other members of the Weaver laboratory, for comments and suggestions; T. Ryan and the UAB ES/Transgenic facility for assistance with blastocyst and embryo injections and the UAB Digestive Diseases Research Development Center (DDRDC) for generation of reporter mice; H. Shen for the provision of LM-OVA; C. Song, H. Turner and M. Blake for technical assistance; and N. LeLievre for editorial assistance. This study was supported in part by a Postdoctoral Fellowship and Career Transition Award from the National Multiple Sclerosis Society (L.E.H.) and by grants from the National Institutes of Health (C.T.W. and A.J.Z.).

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LETTERS

Preserving cell shape under environmental stress

Boaz Cook[1], Robert W. Hardy[1], William B. McConnaughey[2] & Charles S. Zuker[1]

Maintaining cell shape and tone is crucial for the function and survival of cells and tissues. Mechanotransduction relies on the transformation of minuscule mechanical forces into high-fidelity electrical responses[1–3]. When mechanoreceptors are stimulated, mechanically sensitive cation channels open and produce an inward transduction current that depolarizes the cell. For this process to operate effectively, the transduction machinery has to retain integrity and remain unfailingly independent of environmental changes. This is particularly challenging for poikilothermic organisms, where changes in temperature in the environment may impact the function of mechanoreceptor neurons. Thus, we wondered how insects whose habitat might quickly vary over several tens of degrees of temperature manage to maintain highly effective mechanical senses. We screened for *Drosophila* mutants with defective mechanical responses at elevated ambient temperatures, and identified a gene, *spam*, whose role is to protect the mechanosensory organ from massive cellular deformation caused by heat-induced osmotic imbalance. Here we show that Spam protein forms an extracellular shield that guards mechanosensory neurons from environmental insult. Remarkably, heterologously expressed Spam protein also endowed other cells with superb defence against physically and chemically induced deformation. We studied the mechanical impact of Spam coating and show that spam-coated cells are up to ten times stiffer than uncoated controls. Together, these results help explain how poikilothermic organisms preserve the architecture of critical cells during environmental stress, and illustrate an elegant and simple solution to such challenge.

Fly mechanoreceptor neurons (MRNs) are essential for several critical functions such as hearing, proprioception, flight control and touch sensing. Their mis-function leads to uncoordination and loss of mechanoreceptor responses[4,5]. To identify components of the machinery that preserve the functional integrity of the mechanosensory apparatus at high environmental temperatures, we performed a genetic screen for temperature-sensitive uncoordinated flies; we anticipated that loss-of-function mutations in such components may render MRN function highly susceptible to the elevated temperature. Approximately 12,000 ethylmethane-sulphonate-mutagenized homozygous lines[6] were examined for intact locomotor responses at room temperature, but defective behaviour after 1 h at 37 °C. One mutant line, *2649*, had no apparent defects at room temperature, including walking, feeding and flying. However, upon shifting to the restrictive temperature, the flies gradually lost the ability to fly, to stand upside down and to climb walls, until eventually they could only lie and sporadically move their legs, wings and mouthparts in an uncoordinated manner (Supplementary Fig. 1 and Supplementary videos). Genetic mapping and transformation rescue experiments proved that the mechanosensory defects of line 2649 are due to a non-sense mutation in the spacemaker gene (*spam*; see Fig. 1).

Recently, we showed that *spam* encodes an extracellular protein required for creating the intra-rhabdomeral space in the compound eyes of insects with open rhabdom systems[7]. There, Spam provides the extracellular substrate to sustain the precise arrangement of rhabdomeres within each ommatidium[7]. Notably, the other sites of Spam expression are on mechanosensory and chemosensory neurons[8]. To

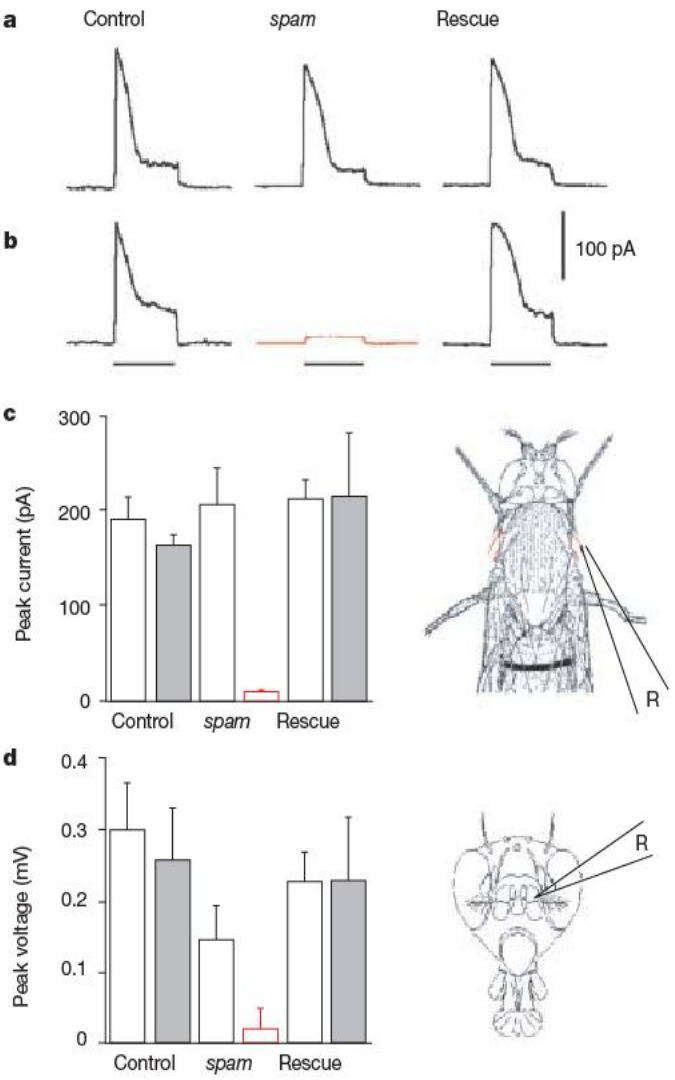

Figure 1 | Effect of heat exposure on the function of mechanoreceptor cells. **a**, **b**, MRN responses from a single voltage-clamped bristle to mechanical stimuli. **a**, (left to right): responses of control (*cn bw* flies), *spam* (*spam/spam* mutants) and rescue flies (*spam* homozygotes expressing a wild-type *spam* transgene). Note normal responses of all three samples at 21 °C. **b**, After incubation for 30 min at 37 °C, responses were abolished in *spam* mutants (red trace). Lines under the traces indicate the duration of 0.3 s of a deflection stimulus of 30 μm. **c**, Summary of peak responses of **a**, **b** (right-hand diagram illustrates the site of recordings). **d**, Summary of peak extracellular voltage responses to antennal rotation (pipette position illustrated on the right-hand diagram). Open columns, 21 °C; hatched columns, responses after 30 min at 37 °C. R, the position of the recording pipette. Error bars, s.d. ($n \geq 6$ for each trial).

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directly examine the impact of loss-of-function mutations in *spam* on mechanosensory transduction, we performed electrophysiological recordings from bristle mechanoreceptors (touch)[9] and antennal chordotonal organs (hearing)[10] from control and mutant flies. We gave sensory bristles calibrated mechanical stimuli while recording transduction currents with a voltage-clamp apparatus. At 21 °C, control flies and *spam* mutants displayed robust inward currents in response to bristle deflections (Fig. 1a–c). In contrast, 30 min of exposure to 37 °C reduced mechanoreceptor response amplitudes in *spam* mutant animals by over 80%. The same heat exposure also nearly abolished all mechanoreceptor antennal responses, while having no significant effect on control flies (Fig. 1d).

Next, we examined the ultrastructure of MRNs in control flies and *spam* mutants at both permissive and non-permissive temperatures. *Drosophila* mechano- and chemosensory neurons house their entire sensory apparatus in a ciliated outer segment that forms the neuronal sensory endings[11]. In MRNs, this outer segment is bathed in an extracellular fluid (lymph) which provides the proper ionic environment for the generation of mechanoreceptor currents[11]. Remarkably, *spam* mutants, but not control flies, experience a dramatic deformation of their MRNs in response to heat treatment: the entire neuronal cytoplasm invades the lymph space, such that the region that normally contained only the cilium and extracellular fluid now becomes filled with cellular material from the MRN cell body (compare Fig. 2a, b and Fig. 2c; see also Supplementary Figs 2 and 4).

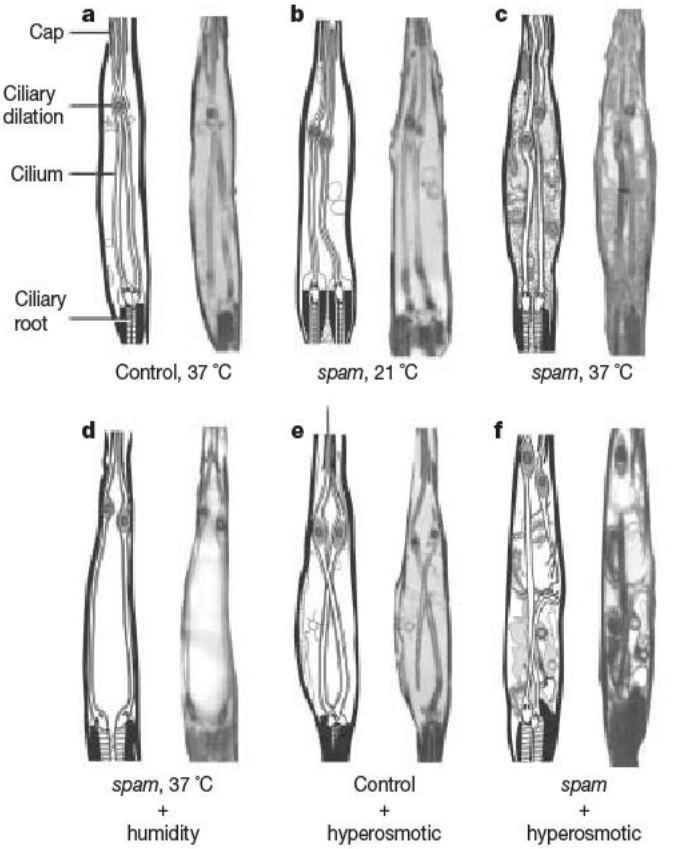

Figure 2 | Mechanoreceptors of *spam* mutants undergo dramatic cellular deformation. Electron micrographs of a typical scolopale MRN in the Johnston's organ. **a**, Control (*cn bw*) flies at 37 °C and (**b**) *spam* homozygous mutants at 21 °C have nearly indistinguishable morphology (equivalent results are observed with *cn bw* flies at 21 °C). However, (**c**) exposure of *spam* flies to 30 min at 37 °C results in major cellular deformation, with the receptor cell cytoplasm expanding to fill the entire scolopale space (importantly, the cells that wrap around the scolopale space are unaffected; data not shown). **d**, Placing *spam* mutants in a high-humidity chamber (greater than 90% relative humidity) prevents the heat-induced deformation. **e**, **f**, *cn bw* control and *spam* flies injected with a hyper-osmotic solution to the abdomen. Only *spam* mutants display dramatic cellular deformation with extensive invasion of the extracellular space. Note that some of the reconstructed electron micrographs show a side view of the scolopale, with only one ciliary root visible (**a** and **e**), whereas all others show a front view, with both ciliary roots visible.

How does exposure to elevated temperatures have such a dramatic effect on the morphology of *spam* MRNs? Changes in molecular thermal motion between 21 °C and 37 °C are too small, and unlikely to account for the phenotype. We therefore considered a prominent secondary effect of heat: water loss by evaporation. To investigate how much water is lost during the heat exposure, we measured the weight of control and mutant flies at intervals of 15 min. All flies lose about 20% of their total weight after 60 min at 37 °C (about 25% of their water content; data not shown), yet only the mutants display the mechanosensory defect. To determine whether the heat-induced deformation of MRN in *spam* mutants is indeed a consequence of water loss, we placed *spam* flies either in a control Petri dish or in a dish at over 90% humidity, and subjected them to 37 °C for 60 min. Notably, only the flies in the dry chamber were affected by heat; exposure to high humidity during the high-temperature treatment completely prevented the manifestation of the mutant phenotype, both morphologically (Fig. 2c, d) and behaviourally (Supplementary material, compare Supplementary Videos 4 and 6). These data demonstrate that the mutant's mechanosensory deficit does not arise from an effect of temperature per se, but is instead triggered by excessive water evaporation at high temperature[12]. Why does water loss lead to deformation of the MRN only in *spam* mutants? We hypothesized that the rapid loss of water from the animal's circulatory system (haemolymph) would increase its osmolarity, leading to an outflow of water from the sensory lymph. The new imbalance between the MRN cytoplasm and the lymph would cause the deformation of the MRN cytosol, which if not contained (as in the absence of Spam protein; see below), would then invade the lymph space. This proposed mechanism anticipates that hypertonic shock to the haemolymph of *spam* mutants, but not wild-type animals, should mimic the effect of high temperature on the morphology and function of MRNs (that is, hypertonic shock should induce a similar osmotic imbalance between the endolymph and the cytoplasm of the MRN). We injected a high-osmolarity solution to the abdomen of *spam* and control flies and prepared them for examination by electron microscopy. As hypothesized, only *spam* flies showed deformation of the MRN (Fig. 2e, f) and loss of mechanosensory responses (Supplementary Fig. 3), substantiating the mechanism of deformation and the role of Spam in maintaining cell shape.

In photoreceptor neurons, Spam is secreted into the interrhabdomeral space, where it forms the extracellular medium that organizes and preserves the separation of rhabdomeres. We reasoned that in mechanoreceptor neurons the role of Spam might be a variation on this theme, perhaps serving as a cellular exoskeleton that provides structural rigidity to the MRN, thus ensuring the preservation of cell shape under environmental stress. This postulate makes two significant predictions. First, Spam protein should be specifically localized within the fly's mechanoreceptor organ, at locations that might be particularly vulnerable to osmotic pressure changes. Second, if Spam functions as a mechanical barrier that protects MRN from deformation, it should be possible to engineer cells that are coated with Spam and make them resistant to osmotic insult and deformation pressures. Indeed, Spam protein concentrates at two specific sites in MRN: one, right at the interface between the MRN cell body and the lymph space, the very domain that collapses at high temperature in mutant animals (see Supplementary Fig. 4d, e); and at a second site close to the ciliary dilation, possibly helping sustain the two ciliary processes at the proper position (Supplementary Fig. 4). To generate cells that are decorated by a layer of Spam, we took advantage of Spam's ability to directly bind the membrane receptor Prominin[7]. Therefore, *Drosophila* tissue culture cells expressing and secreting Spam were incubated with GFP-labelled cells transfected with Prominin. As expected, secreted Spam specifically decorated the surface of Prominin-expressing cells; to identify those

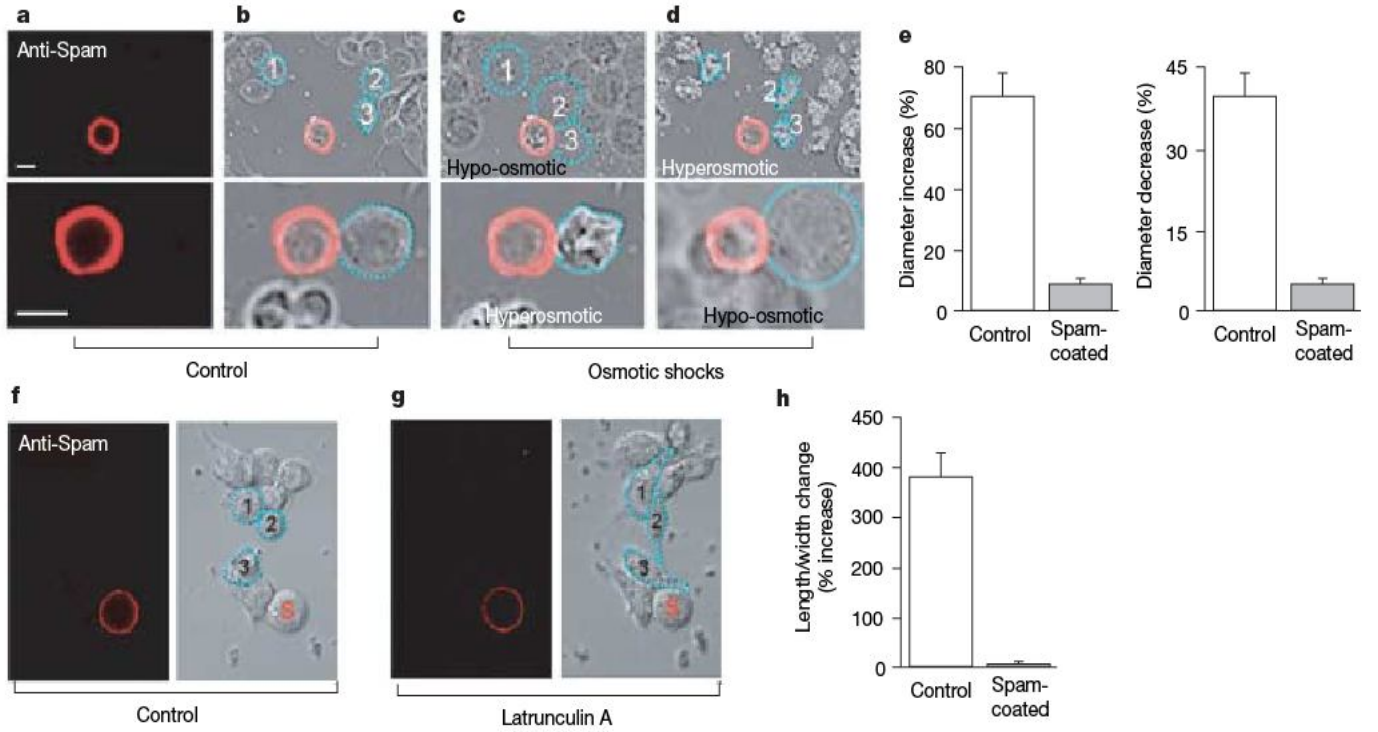

Figure 3 | Spam coating prevents cell deformation induced by osmotic or chemical manipulation. **a–d**, Kc tissue culture cells transfected with Prom and Spam were stained for Spam surface labelling (red) using anti-Spam antibodies on unpermeabilized, intact cells. Spam-coated (**a**) and uncoated cells were then subjected to osmotic shock. **b–d**, Upper panels show low-magnification images of cells before and after sequential hypo- and hyper-osmotic shock; lower panels show similar cells at higher magnification but this time after sequential hyper- and then hypo-osmotic shock (reverse order). Hypo-osmotic shock causes dramatic swelling of uncoated cells, whereas hyper-osmotic treatment of the same preparation leads to extreme shrinking. Notably, the Spam-coated cell remains largely unaffected by both treatments. All cells that showed a continuous layer of Spam coating (a layer thicker than 0.5 μm with no apparent gaps) showed no significant shape changes in response to the osmotic shocks ($n = 11$), whereas all uncoated cells displayed severe changes in size and shape ($n > 150$). **e**, Increases (left) and decreases (right) in cell size after hypotonic or hypertonic shock; control, $n \geq 24$; spam-coated, $n \geq 8$; error bars, s.e.m. **f**, Spam-coated (red) and uncoated cells were incubated for 90 min with latrunculin A. **g**, As expected, control cells undergo dramatic changes in cell shape[13]. In contrast, the spam-coated cell (S) remains unaltered. Blue dots and numbers delineate the shape of three sample uncoated cells before (**f**) and after (**g**) treatment. Images were captured with epifluorescence and Nomarski interference contrast. **h**, Changes in cell shape (defined as changes in the ratio of cell length over width) after treatment with latrunculin in Spam-coated ($n = 9$) and control uncoated cells ($n = 27$); error bars, s.e.m.

cells that are entirely (or nearly completely) coated, we performed immunofluorescent staining with anti-Spam antibodies. We induced cellular deformation by subjecting control and coated cells to hyper- and hypo-osmotic solutions. As predicted, control cells undergo significant swelling after hypo-osmotic shock, and severe shrinking in the presence of hyper-osmotic solutions (Fig. 3a–e). In contrast, coated cells were largely resistant to these treatments and showed only minor changes in shape and size (not surprisingly, poorly coated cells were indistinguishable from controls; data not shown). Next, we examined the impact of Spam on chemically induced changes in cell shape[13]. We subjected control cells to latrunculin A and elicited dramatic changes in cell morphology (Fig. 3f–h). However, Spam-coated cells retained their normal spherical shape, even after extensive actin remodelling resulting from the treatment with latrunculin A (see Methods). Collectively, these studies demonstrate that Spam coating of the plasma membrane endows cells with exquisite protection against osmotically and chemically induced transformations in cell shape.

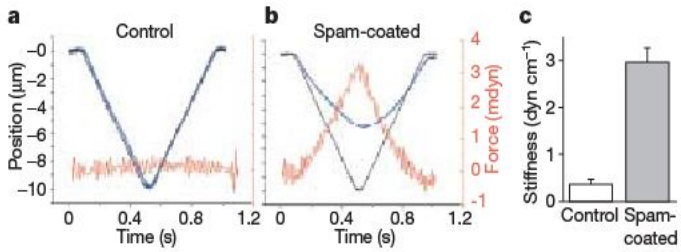

Figure 4 | Mechanical impact of Spam coating. To measure the stiffness of Spam-coated cells, (**a**) control or (**b**) Spam-coated tissue culture cells were subjected to a mechanical indentation assay[14]. To reduce the contribution of the cytoskeleton to cell stiffness (and thereby reveal the effect of Spam coating more effectively), samples were pre-treated with cytochalasin D as previously described[15]. **a**, **b**, Comparison of the position over time of the motor that moves the probe assembly (black trace) versus the position of the stylus that indents the cell (blue trace). The difference between the two curves at a given time is due to the cell's resistance to indentation. The force applied by the cell against the probe is proportional to this difference and is shown in red. **c**, Stiffness (cell resistance force per unit indentation) was calculated as described[14]. A minimum of nine individual cells were examined for each experiment; error bars, s.e.m. Control cells without cytochalasin D are presented in Supplementary Fig. 5.

How robust are Spam-treated cells? We directly examined the stiffness of Spam-coated and control cells by measuring their mechanical properties. In these experiments, a glass filament of known bending constant is continuously pressed against the cell by a linear piezoelectric drive[14] (Fig. 4a, b). The force applied to the tip of the probe by the resistance of the cell to indentation is then calculated by optically measuring the bending of the glass probe. The major source of stiffness in cells is the actin cytoskeleton[15]. Therefore, to eliminate the contribution of the cytoskeleton and explore the specific effect of Spam, experimental and control samples were first treated with cytochalasin D for 120 min. The results (Fig. 4c) demonstrate that Spam-coated cells exhibit stiffness that is approximately ten times that of control cells.

Together, these studies have revealed a remarkable solution to the problem of maintaining cellular integrity and structure under duress. They also provide a salient example of evolution using the same protein to satisfy two very different needs: the building of compound eyes in open rhabdom systems[7,16], and the preservation of cell shape

in mechano- and chemoreceptor organs. Interestingly, both entail the production and assemblage of a rigid substrate, thus highlighting the fundamental role of Spam in tissue morphogenesis (in one scenario to ensure the partitioning and maintenance of the rhabdomere complex, and in the other to guarantee the mechanical integrity of sensory neurons). Finally, it is worth noting that the ability to assemble a 'cell wall' surrounding an animal cell may provide the foundation for important applications in cell engineering, where resistance to osmotic pressures may be warranted, or where preservation of cell and tissue structure (or tone) may be needed.

METHODS SUMMARY

Fly stocks. An isogenized *cn bw* stock was used as control in all experiments. The *spam* line was isolated from the Zuker collection[6] and the rescue was done using hs-gal4 driving UAS-spam[7].

Electrophysiology. Single bristle current recordings were performed as described earlier[9]. Voltage changes resulting from activation of Johnston's organ were monitored by inserting a glass pipette (2 M KCl, approximately 10 MΩ) into the second antennal segment. Mechanical stimulation was delivered by a stream of air that was directed at the arista, causing a rotation of the third segment for the duration of air flow.

***In vivo* osmotic manipulation.** Flies were glued ventral side up and manually injected by using a glass pipette with a tip of 20–40 μm. After 10 min, tissue was prepared for analysis as described under Electron microscopy.

Electron microscopy. Heads of 7- to 10-day-old flies were fixed and sectioned exactly as previously described[7]. A series of coronal sections (100–200 nm per section) through the antennal second segment was obtained. The entire scolopale was reconstructed from overlapping sections using Adobe imaging software.

Tissue culture. Kc cells[13] were transfected with combinations of pTub-GAL4 and pUAST-spacemaker, pUAST-prominin and pUAST-GFP, as previously described[7]. Spam coating was detected *in vivo* by using its specific antibody mAb21A6[17]. Hypo-osmotic shock was induced by diluting the growth medium 1:5× with distilled water; hyper-osmotic conditions were obtained by adding 50 μl of 5 M NaCl to 1.25 ml of growth media. Latrunculin A (Sigma) was used at a final concentration of 0.2 μM (ref. 18).

Cell indentation assay. KC cells co-transfected with pTub-GAL4, pUAST-spacemaker and pUAST-prominin were treated with 2 μM cytochalasin D for at least 100 min, and the Spam-coated cells identified by labelling with anti-Spam antibodies. Indentation tests were performed on control and Spam-coated cells as previously described[14].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 3 September; accepted 20 December 2007.
Published online 24 February 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We particularly thank E. L. Elson for his help and hospitality in the cell poking studies. We are also indebted to A. Zelhof for advice, materials and reagents, and T. Avidor-Reiss for his help with the immunolocalization of Spam. We thank T. Meerloo for help with immunogold labelling, and A. Becker for help with tissue culture and transfections. We thank N. Ryba, A. Kiger and members of the Zuker laboratory for comments. C.S.Z. is an investigator of the Howard Hughes Medical Institute.

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LETTERS

Control of chromosome stability by the β-TrCP–REST–Mad2 axis

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REST/NRSF (repressor-element-1-silencing transcription factor/neuron-restrictive silencing factor) negatively regulates the transcription of genes containing RE1 sites[1,2]. REST is expressed in non-neuronal cells and stem/progenitor neuronal cells, in which it inhibits the expression of neuron-specific genes. Overexpression of REST is frequently found in human medulloblastomas and neuroblastomas[3–7], in which it is thought to maintain the stem character of tumour cells. Neural stem cells forced to express REST and c-Myc fail to differentiate and give rise to tumours in the mouse cerebellum[3]. Expression of a splice variant of REST that lacks the carboxy terminus has been associated with neuronal tumours and small-cell lung carcinomas[8–10], and a frameshift mutant (REST-FS), which is also truncated at the C terminus, has oncogenic properties[11]. Here we show, by using an unbiased screen, that REST is an interactor of the F-box protein β-TrCP. REST is degraded by means of the ubiquitin ligase $SCF^{β\text{-}TrCP}$ during the G2 phase of the cell cycle to allow transcriptional derepression of *Mad2*, an essential component of the spindle assembly checkpoint. The expression in cultured cells of a stable REST mutant, which is unable to bind β-TrCP, inhibited *Mad2* expression and resulted in a phenotype analogous to that observed in $Mad2^{+/-}$ cells. In particular, we observed defects that were consistent with faulty activation of the spindle checkpoint, such as shortened mitosis, premature sister-chromatid separation, chromosome bridges and mis-segregation in anaphase, tetraploidy, and faster mitotic slippage in the presence of a spindle inhibitor. An indistinguishable phenotype was observed by expressing the oncogenic REST-FS mutant[11], which does not bind β-TrCP. Thus, $SCF^{β\text{-}TrCP}$-dependent degradation of REST during G2 permits the optimal activation of the spindle checkpoint, and consequently it is required for the fidelity of mitosis. The high levels of REST or its truncated variants found in certain human tumours may contribute to cellular transformation by promoting genomic instability.

F-box proteins are the substrate-recognition subunits of SCF (SKP1–CUL1–F-box protein) ubiquitin ligases, providing specificity to ubiquitin conjugation reactions[12,13]. Mammals express two paralogues of the F-box protein β-TrCP (β-TrCP1 and β-TrCP2) that are biochemically indistinguishable; we shall therefore use β-TrCP to refer to both, unless otherwise specified.

To identify substrates of the $SCF^{β\text{-}TrCP}$ ubiquitin ligase, we used an immunoaffinity/enzymatic assay followed by mass spectrometry analysis[14,15]. In two independent purifications, peptides corresponding to REST were identified. The interaction between REST and β-TrCP suggested that $SCF^{β\text{-}TrCP}$ is the ubiquitin ligase targeting REST for degradation. To investigate the specificity of this binding, we screened 16 F-box proteins as well as two related proteins, CDH1 and CDC20. β-TrCP1 and β-TrCP2 were the only proteins that immunoprecipitated together with endogenous REST (Fig. 1a and data not shown). Interaction between endogenous β-TrCP1 and REST was also observed (Supplementary Fig. 1a).

Most proteins recognized by β-TrCP contain a DSGXXS degron in which the serine residues are phosphorylated, allowing binding to β-TrCP[16]. REST has a similar motif at the C terminus in which the first serine residue is replaced by glutamic acid, in an analogous manner to other known β-TrCP substrates (Supplementary Fig. 2a). Supplementary Fig. 3 shows that this sequence fits with low energy into the three-dimensional structural space of the β-TrCP substrate-binding surface, similarly to a phospho-peptide corresponding to the degron of β-catenin, a well-characterized substrate of β-TrCP[17].

We generated a number of human REST mutants (all with haemagglutinin epitope (HA) tags), in which Glu 1009 and/or Ser 1013 were mutated to Ala (Supplementary Fig. 2b), expressed them in HEK-293T cells, and immunoprecipitated them with anti-HA resin. Whereas wild-type REST efficiently immunoprecipitated endogenous β-TrCP1, the REST(E1009A), REST(S1013A) and REST (E1009A/S1013A) mutants did not (Fig. 1b and Supplementary Fig. 1b), showing that Glu 1009 and Ser 1013 are required for binding to β-TrCP. Accordingly, in comparison with wild-type REST, the half-lives of REST mutants were increased in HEK-293T cells (Fig. 1c).

Because $SCF^{β\text{-}TrCP}$ mediates the ubiquitination of several proteins in specific phases of the cell cycle[12,15,18–20], we analysed the expression of REST during the cell cycle. When HeLa cells were released from a G1/S block, REST protein levels decreased in G2, at a time when the levels of cyclin A and Emi1, which are both degraded in early mitosis, were still elevated (Fig. 1d). Similar oscillations in REST expression were observed with different synchronization methods and cell types, including HCT116, U-2OS and human diploid IMR-90 fibroblasts (Supplementary Fig. 4a, b and data not shown). The proteasome inhibitor MG132 prevented the disappearance of REST in HeLa and HCT116 cells arrested in prometaphase by a spindle poison (Supplementary Fig. 5a), showing that REST degradation is mediated by the proteasome and that this degradation persists during spindle checkpoint activation. Accordingly, in contrast with wild-type REST, REST(E1009A/S1013A) is stable in prometaphase cells (Supplementary Fig. 6a, b).

MG132-treated prometaphase cells accumulated phosphorylated REST (Supplementary Fig. 5b). Moreover, REST, but not REST (E1009A/S1013A), immunopurified from prometaphase cells was ubiquitinated *in vitro* in the presence of β-TrCP (but not FBXW8) (Fig. 1e and Supplementary Fig. 5c). Finally, incubation with

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Of the 37 proteins analysed, only the levels of Mad2 were altered in cells expressing REST(E1009A/S1013A) (Fig. 2a, and data not shown). Lower levels of Mad2 were also observed when the stable REST mutant was expressed in IMR-90 fibroblasts (Supplementary Fig. 7) and NIH 3T3 cells (data not shown). Mad2 is a crucial component of the spindle checkpoint, inhibiting the anaphase-promoting complex to prevent sister-chromatid separation until microtubules radiating from the spindle poles have been attached to all kinetochores[21]. Northern blot analysis showed downregulation of *Mad2* mRNA in REST(E1009A/S1013A)-expressing cells (Fig. 2b). Analysis of the *Mad2* genomic sequence showed several putative RE1 sites. Chromatin immunoprecipitation analysis confirmed *in vivo* binding of endogenous REST to the *Mad2* promoter (Fig. 2c). In addition, a human *Mad2* genomic fragment containing an RE1 site (position 26–46 relative to the transcription start site), but not one containing a deletion in the RE1 site, conferred REST responsiveness to a luciferase reporter after the transient transfection of U-2OS or IMR-90 cells (Fig. 2d and Supplementary Fig. 8). Dominant-negative

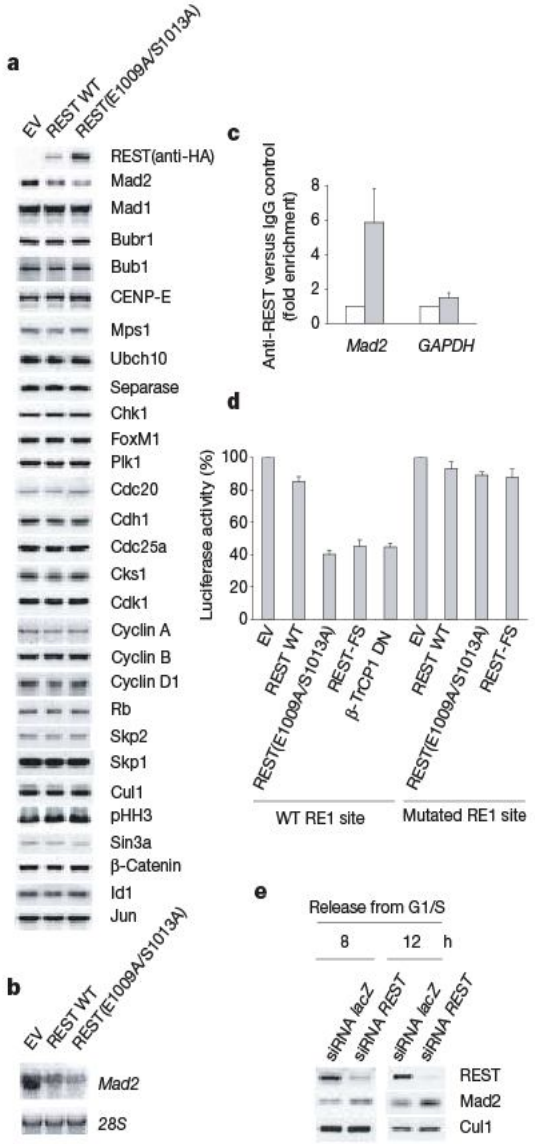

Figure 2 | *Mad2* is a transcriptional target of REST. **a**, U-2OS cells were infected either with an empty lentivirus (EV) or with lentiviruses expressing HA-tagged wild-type REST or HA-tagged REST(E1009/S1013A). After treatment with nocodazole for 15 h, mitotic cells were harvested and analysed by immunoblotting for the indicated proteins. **b**, *Mad2* mRNA was assessed by northern blotting in U-2OS cells treated as in **a**. **c**, Chromatin immunoprecipitation (ChIP) assay with an anti-REST antibody (filled columns) in U-2OS cells. Quantitative real-time PCR amplifications were performed with primers surrounding the RE1 site in the *Mad2* promoter. The value given for the amount of PCR product present from ChIP with control IgG (open columns) was set as 1. GAPDH primers were used as a negative control. **d**, U-2OS cells were transfected with an empty vector (EV), HA-tagged REST proteins or a dominant-negative β-TrCP1 mutant (FLAG–β-TrCP1 DN) together with a luciferase reporter linked to a *Mad2* genomic fragment containing either a wild-type (WT) or a mutated RE1 site. Prometaphase cells were collected and the relative luciferase signal was quantified. The value given for luciferase activity in EV-transfected cells was set at 100%. **e**, IMR-90 cells were transfected twice with siRNA molecules to a non-relevant mRNA (*lacZ*) or to *REST* mRNA and synchronized in G2 by release from an aphidicolin block for the indicated durations[30]. Cells were then lysed and immunoblotted. Where present, error bars represent s.d. ($n = 3$).

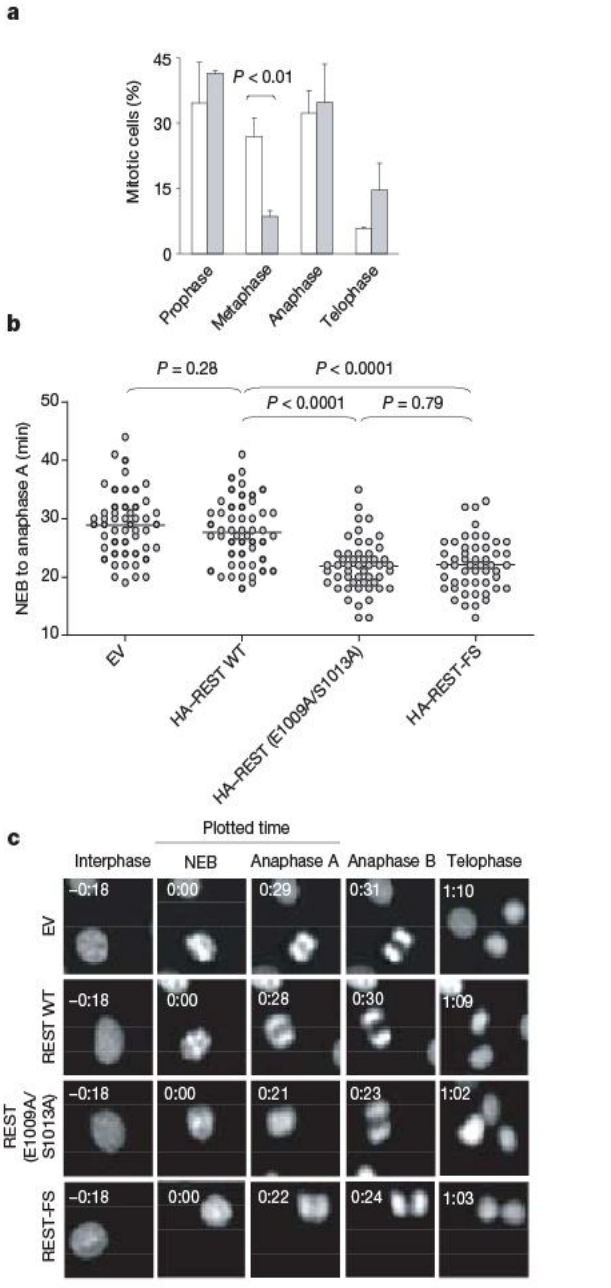

Figure 3 | Failure to degrade REST causes defects in the mitotic checkpoint. **a**, HCT116 cells were infected either with an empty lentivirus (EV, open columns) or with a lentivirus expressing REST(E1009/S1013A) (filled columns). At 48 h after infection, cells were fixed and stained with 4,6-diamidino-2-phenylindole and an anti-α-tubulin antibody to reveal DNA and the mitotic spindle, respectively. Error bars represent s.d. ($n = 3$). **b**, NIH 3T3 cells stably transfected with enhanced green fluorescent protein-labelled histone H2B were infected with an empty lentivirus or with lentiviruses expressing the indicated HA-tagged proteins. The average time from nuclear envelope breakdown (NEB) to anaphase onset was measured by time-lapse microscopy. Each symbol in the scatter plot represents a single cell. **c**, Representative fluorescence videomicroscopy series from **b**; numbers in the top left are times (h:min).

β-TrCP[22], which stabilizes endogenous REST (data not shown), inhibited the activity of the *Mad2* promoter-driven luciferase reporter (Fig. 2d). Importantly, depletion of REST in G2 IMR-90 cells induced an increase in Mad2 levels (Fig. 2e).

These results indicate that *Mad2* is a direct and physiologically relevant transcriptional target of REST. Consistent with this notion, *Mad2* expression (both at the mRNA and protein level) is inversely proportional to REST protein levels during the progression of cells through G2 (Supplementary Fig. 4b, c).

Deletion of a single *Mad2* allele in mouse embryonic fibroblasts or human HCT116 cancer cells results in a defective mitotic checkpoint[23]. To study whether failure to degrade REST in G2 also affects the spindle checkpoint, we analysed HCT116 cells (which have a relatively stable karyotype) expressing HA-tagged wild-type REST or HA-tagged REST(E1009A/S1013A). As expected, wild-type REST was degraded in G2, whereas REST(E1009A/S1013A) was stable in G2 (Supplementary Fig. 9). Cells expressing REST (E1009A/S1013A) showed a decreased percentage of metaphases (Fig. 3a). Because this effect might have been due to a faster progression through metaphase, we analysed mitotic progression by time-lapse microscopy. The average time from nuclear envelope breakdown to anaphase onset was decreased in cells expressing REST(E1009A/S1013A) in comparison with control cells (Fig. 3b, c and Supplementary Fig. 10). Moreover, expression of the stable REST mutant increased the number of lagging chromosomes and chromosome bridges in anaphase (Fig. 4a) and the appearance of tetraploidy (11/61 versus 0/61 in control cells), as scored in metaphase spreads from two different experiments. More than 8% of cells expressing the stable REST mutant (6/71) displayed prematurely separated sister chromatids, in contrast with 1.4% (1/71) in control cells (Fig. 4b). Finally, cells expressing the stable REST mutant showed a decrease in the mitotic index in the presence of a spindle poison, despite the fact that they entered into mitosis with normal kinetics (Fig. 4c and Supplementary Fig. 11), suggesting an increased rate of mitotic slippage and adaptation to the spindle checkpoint.

These phenotypes indicate that in cells expressing the stable REST mutant, anaphase proceeds faster and in the absence of complete and accurate chromosome–microtubule attachment. Premature anaphase and chromosome aberrations are hallmarks of the defective spindle checkpoint observed in $Mad2^{+/-}$ cells[23].

We predicted that REST-FS, an oncogenic frame-shift mutant from a colon cancer cell line[11], would be stabilized because it lacks the β-TrCP degron. Indeed, we found that REST-FS did not bind β-TrCP and was stable in G2 HCT116 cells (Supplementary Figs 1b and 12a). Expression of REST-FS in U-2OS cells caused a decrease in *Mad2* mRNA and Mad2 protein (Supplementary Fig. 12b, c) and a decrease in the activity of a *Mad2* promoter-driven luciferase reporter (Fig. 2d), similarly to the expression of REST(E1009A/S1013A). Finally, the mitotic phenotypes induced by the expression of REST-FS (Figs 3b, c and 4) were indistinguishable from those caused by REST(E1009A/S1013A).

We show here that β-TrCP-mediated degradation of REST in G2 is necessary for the optimal expression of *Mad2*. Failure to degrade REST produces a deficient spindle checkpoint and consequent chromosome instability. REST is expressed in non-neuronal cells and stem/progenitor neural cells, in which it inhibits neuronal differentiation by blocking the expression of neuron-specific genes[2,24]. The transition from embryonic stem cell to stem/progenitor neuronal cell requires the proteasome-mediated degradation of REST[24]. Our study suggests that REST proteolysis must be accurately controlled to avoid subjecting neuronal tissues to cancer risk. In fact, increased levels of REST resulting from overproduction and/or C-terminal truncations, as observed in human neuronal tumours[3–7,10], would both inhibit differentiation and generate chromosomal instability, two mechanisms that contribute to tumour development. Although REST has oncogenic properties in neuronal cells, the reduction of REST expression in certain non-neuronal tumours suggests a tumour suppressor role for REST and a function in the neuroendocrine phenotype in some of these lesions[11,25]. C-terminally truncated REST variants are also observed in non-neuronal tumours[8–11]. Our study suggests that these stable REST variants may contribute to cell

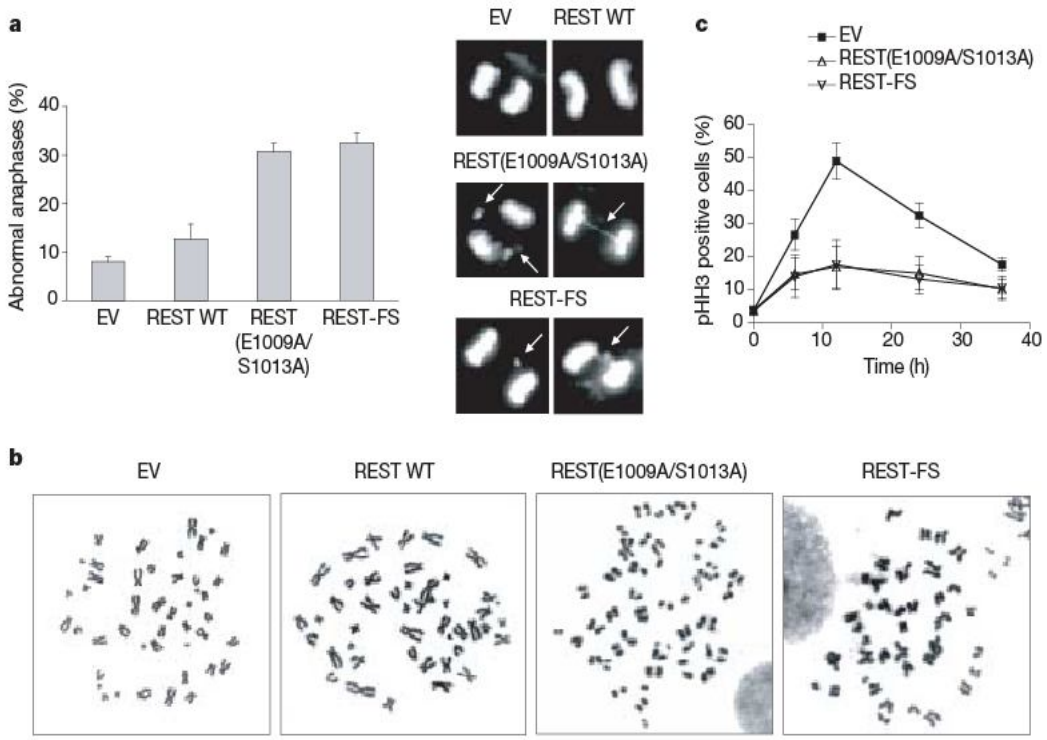

Figure 4 | Expression of a stable REST mutant or oncogenic REST-FS leads to chromosomal instability. **a**, Left: percentage of aberrant anaphases in HCT116 cells infected as in Fig. 3b. Right: representative pictures. Arrows point to lagging chromosomes and chromosome bridges. **b**, Premature sister-chromatid separation in cells expressing stable REST. Panels show representative metaphase spreads in HCT116 cells infected as in Fig. 3b. **c**, Nocodazole was added for the indicated durations to HCT116 cells infected as in Fig. 3b. Cells were stained with an anti-phospho-HH3 (pHH3) antibody to quantify their mitotic index. Where present, error bars represent s.d. ($n = 3$).

transformation by promoting aneuploidy[26] and genetic instability in both neuronal and non-neuronal tissues.

METHODS SUMMARY

Biochemical methods. Extract preparation, immunoprecipitation and immunoblotting were as described previously[14,15,18].

Transient transfections and lentivirus-mediated gene transfer. HEK-293T cells were transfected by using calcium phosphate. U-2OS cells were transfected with the use of FuGENE-6 reagent (Roche). For lentivirus-mediated gene transfer, HEK-293T cells were co-transfected with pTRIP-PGK together with packaging vectors. At 48 h after transfection, virus-containing medium was collected and supplemented with 8 $\mu g\,ml^{-1}$ Polybrene (Sigma). Cells were then infected with the viral supernatant for 6 h.

Transcription analyses. RNA was extracted by using the RNeasy Kit (Qiagen). cDNA synthesis was performed with Superscript III (Invitrogen). Quantitative real-time PCR analysis was performed in accordance with standard procedures, using SYBR Green mix (Bio-Rad). Mad2 primer sequences were reported previously[27]. Control ARPP P0 primers sequences were 5′-GCACTGGAAGTCCAACTACTTC-3′ and 5′-TGAGGTCCTCCTTGGTGAACAC-3′. Northern blotting was performed as described[28]. ^{32}P-labelled human full-length *Mad2* complementary DNA was used as a probe to detect *Mad2* mRNA. For luciferase assays, U-2OS cells were transfected in a 4:2:1 ratio with HA-tagged REST constructs, luciferase reporter plasmid (pGL3-Mad2)[27] and pCMV-β-galactosidase. Luciferase activity was measured with the Luciferase Reporter Assay System (Promega), and relative luciferase activities were normalized to *lacZ*.

Chromatin immunoprecipitations. Chromatin immunoprecipitations were conducted as described previously[29]. Mad2 primer sequences were as reported previously[27]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primer sequences were 5′-TCCACCACCCTGTTGCTGTA-3′ and 5′-ACCACAGTCCATGCCATCAC-3′.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 1 November 2007; accepted 9 January 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank V. D'Angiolella, S. Ge, L. Gnatovskiy, J. Staveroski and N. E. Sherman for their contributions to this work; P. Jallepalli and J. Skaar for suggestions and/or critically reading the manuscript; S. Elledge and T. Westbrook for communicating results before publication; and the T. C. Hsu Molecular Cytogenics Core. M.P. is grateful to T. M. Thor for continuous support. D.G. is grateful to R. Dolce and L. Guardavaccaro. This work was supported by an Emerald Foundation grant to D.G., a fellowship from Provincia di Benevento to D.G., American–Italian Cancer Foundation fellowships to D.G., N.V.D. and A.P., and grants from the National Institutes of Health to S.C. and M.P.

Author Contributions D.G. performed and planned all experiments (except chromosome analysis in Fig. 4b, which was performed by A.S.M. and S.C, and the β-TrCP immunopurifications, which were performed by N.V.D. and A.P.) and helped to write the manuscript. M.P. coordinated the study, oversaw the results and wrote the manuscript. D.F. contributed to time-lapse experiments. E.H. provided reagents and suggestions. T.C. developed the interaction models. A.L. and A.I. performed unpublished experiments to analyse stem cell differentiation. All authors discussed the results and commented on the manuscript.

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LETTERS

$SCF^{\beta\text{-}TRCP}$ controls oncogenic transformation and neural differentiation through REST degradation

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The RE1-silencing transcription factor (REST, also known as NRSF) is a master repressor of neuronal gene expression and neuronal programmes in non-neuronal lineages[1–3]. Recently, REST was identified as a human tumour suppressor in epithelial tissues[4], suggesting that its regulation may have important physiological and pathological consequences. However, the pathways controlling REST have yet to be elucidated. Here we show that REST is regulated by ubiquitin-mediated proteolysis, and use an RNA interference (RNAi) screen to identify a Skp1-Cul1-F-box protein complex containing the F-box protein β-TRCP ($SCF^{\beta\text{-}TRCP}$) as an E3 ubiquitin ligase responsible for REST degradation. β-TRCP binds and ubiquitinates REST and controls its stability through a conserved phospho-degron. During neural differentiation, REST is degraded in a β-TRCP-dependent manner. β-TRCP is required for proper neural differentiation only in the presence of REST, indicating that β-TRCP facilitates this process through degradation of REST. Conversely, failure to degrade REST attenuates differentiation. Furthermore, we find that β-TRCP overexpression, which is common in human epithelial cancers, causes oncogenic transformation of human mammary epithelial cells and that this pathogenic function requires REST degradation. Thus, REST is a key target in β-TRCP-driven transformation and the β-TRCP–REST axis is a new regulatory pathway controlling neurogenesis.

REST levels decline during differentiation of embryonic stem cells to neural stem and progenitor cells[5], consistent with a role for REST in restraining neuronal gene expression programmes. This decrease results from a threefold reduction in REST half-life (Fig. 1a), suggesting that a regulatory pathway controls REST degradation during early neural differentiation. To determine whether ubiquitination is involved, REST was evaluated for ubiquitin modification *in vivo*. Immunoprecipitation of HA-ubiquitin revealed slower migrating species of REST, suggestive of polyubiquitination (Fig. 1b, lane 3). REST also precipitated with an HA-ubiquitin mutant lacking all lysines except K48 (Fig. 1b, lane 4), suggesting that REST is K48 polyubiquitinated which promotes degradation.

To search for the E3 ubiquitin ligase for REST, we began with the SCF superfamily of ligases[6]. Each SCF family contains a unique Cullin scaffold that is required for ligase function. Notably, co-expression of a dominant negative Cullin-1 (Cul1) mutant resulted in a dramatic increase (11-fold) in REST levels (Supplementary Figure 1b), indicating that one or more Cul1-containing ligases negatively regulate REST abundance.

F-box proteins act as substrate receptors for the SCF[7,8]. To determine which F-box proteins are required for REST turnover, we established a system for monitoring REST abundance in a high-throughput manner using an mRFP-REST fusion protein. Like endogenous REST, mRFP-REST was unstable, and its abundance increased upon inhibition of Cul1 (Supplementary Fig. 2a). To identify the F-box proteins regulating REST, individual short interfering RNA (siRNAs) targeting each F-box protein (four siRNAs per gene) were co-transfected with a plasmid encoding mRFP-REST, and changes in cellular fluorescence were monitored by flow cytometry (Supplementary Fig. 2b). siRNAs that increased fluorescence more than two standard deviations from the mean were re-tested in triplicate for their effects on both mRFP and mRFP-REST to identify siRNAs that specifically alter REST stability (Fig. 1c). This approach identified FBW4 and β-TRCP2. Notably, multiple siRNAs targeting additional sequences within FBW4 and β-TRCP2 also increased mRFP-REST abundance (Fig. 1d), confirming the specificity of the siRNAs. Supporting this conclusion, co-expression of a dominant negative β-TRCP mutant (lacking the F-box) also increased REST levels (Supplementary Fig. 4a, b).

β-TRCP2 and FBW4 may control REST abundance by direct ubiquitination of REST or by modulating upstream regulators of REST. β-TRCP2, but not FBW4, was capable of binding REST in cells (Fig. 1e), suggesting that FBW4-mediated regulation is indirect. The highly homologous β-TRCP1 also interacted with REST (Fig. 1e and Supplementary Fig. 3a), consistent with previous reports that β-TRCP1 and β-TRCP2 have similar substrate specificities and frequently function redundantly[9,10]. Importantly, endogenous β-TRCP and REST interact in cells (Supplementary Fig. 3b), and REST was polyubiquitinated by $SCF^{\beta\text{-}TRCP1}$ *in vitro* (Fig. 1f), suggesting that $SCF^{\beta\text{-}TRCP}$ regulates REST by direct ubiquitination. In agreement, stable expression of short-hairpin RNAs (shRNAs) targeting β-TRCP1 and β-TRCP2 both in human mammary epithelial cells (HMECs) and NIH3T3 cells resulted in a moderate but reproducible increase in REST protein abundance and half-life (Fig. 1g, lanes 2 and 3, and Supplementary Fig. 4c), indicating that endogenous REST is regulated by β-TRCP. These data indicate that $SCF^{\beta\text{-}TRCP}$ controls REST by ubiquitin-mediated destabilization.

$SCF^{\beta\text{-}TRCP}$ binds substrates in a phosphorylation-dependent manner[6,10–14]. Consistent with this, λ-phosphatase treatment abolished the interaction between REST and β-TRCP, and this was prevented by λ-phosphatase inhibitors (Fig. 2a). Notably, a dominant negative frame-shift mutant of REST found in human colon cancer cells[4] failed to interact with β-TRCP and exhibited substantially increased stability in cells (Supplementary Fig. 6a), indicating that the carboxy-terminal half of REST is required for β-TRCP recognition. Analysis of this region revealed a sequence highly similar to the phospho-degron

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found in Cdc25A, a well-documented β-TRCP-substrate[11,12] (Fig. 2b). This putative degron includes a conserved DpSG motif that constitutes a critical interaction element within phospho-degrons for β-TRCP[15]. Mass spectrometry was used to examine phosphorylation of REST within this region. To enable tryptic digestion of the peptide of interest, a N1022R substitution was introduced into REST that does not alter interaction with β-TRCP or protein stability in cells (Supplementary Fig. 5a, b). His-tagged REST^{N1022R} was co-expressed with dominant-negative Cul1 in 293T cells and purified under denaturing conditions (Supplementary Fig. 5c). Analysis of phosphopeptides in REST^{N1022R} demonstrated that S1027 and S1030 within the MSEGSDDSGLHG-ARPVPQESSR peptide are phosphorylated both singly and in combination (Supplementary Fig. 5c–g).

To test the ability of the candidate REST-degron to interact with β-TRCP, peptides spanning the degron were synthesized with phosphates at serines 1024, 1027 and 1030 alone or in combination. Individual serine-phosphorylation facilitated weak (S1030) or no interaction (S1024 or S1027) with β-TRCP (Fig. 2d and Supplementary Fig. 7) whereas peptides phosphorylated in combination at S1027+S1030 or S1024+S1027+S1030 associated with β-TRCP (but not FBW4) with an efficiency comparable to the well-established IκB phospho-degron peptide (Fig. 2d and Supplementary Fig. 7). Mutation of each serine to alanine in the context of full-length REST resulted in decreased binding to β-TRCP, and combined mutation of these critical serines completely abrogated the interaction with β-TRCP (Fig. 2c and Supplementary Fig. 6b). Notably, degron-mutant REST was substantially more stable than wild-type REST in cells (Fig. 2e). These data support the hypothesis that phosphorylation of the REST degron primes ubiquitination by SCF$^{β\text{-TRCP}}$, thereby promoting REST degradation.

The role of β-TRCP in degradation of the REST tumour suppressor predicts that β-TRCP overproduction might transform human cells. To examine this prediction, HMECs stably expressing

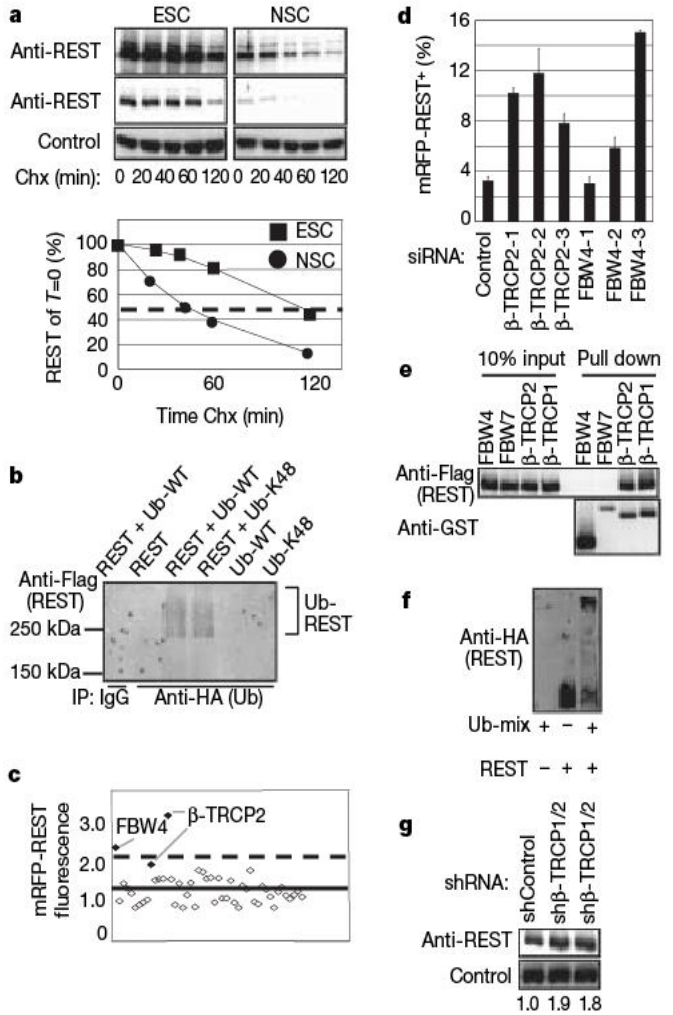

Figure 1 | Identification of β-TRCP and FBW4 ubiquitin ligases as regulators of REST stability. **a**, Embryonic stem cells (ESC) or neural stem cells (NSC) were examined for REST protein half-life in a cycloheximide (Chx) time course. Quantification of relative REST levels in lower panel (dashed line denotes half-life). **b**, 293T cells were transfected with plasmids expressing Flag-REST, HA-ubiquitin and/or HA-ubiquitin-K48 as indicated, immunoprecipitated with HA-specific antibodies or control IgG, and analysed by anti-Flag immunoblot. **c**, siRNA screen for regulators of mRFP-REST (see Supplementary Information for details). **d**, siRNAs targeting β-TRCP2 or FBW4 sequences independent from library-derived siRNAs were tested for effects on mRFP-REST fluorescence ($n = 3$, error bars ± s.d.). **e**, Co-immunoprecipitation of GST-F-box fusion proteins and Flag-REST from mammalian cells. **f**, *In vitro* ubiquitination of HA-REST by SCF$^{β\text{-TRCP}}$ (see Supplementary Information for details). **g**, Human mammary epithelial cells expressing control shRNA or shRNA-targeting human β-TRCP1 and β-TRCP2 were analysed by anti-REST immunoblot. Two independent infections with β-TRCP-shRNA are shown. Quantification of relative REST levels is shown below each lane.

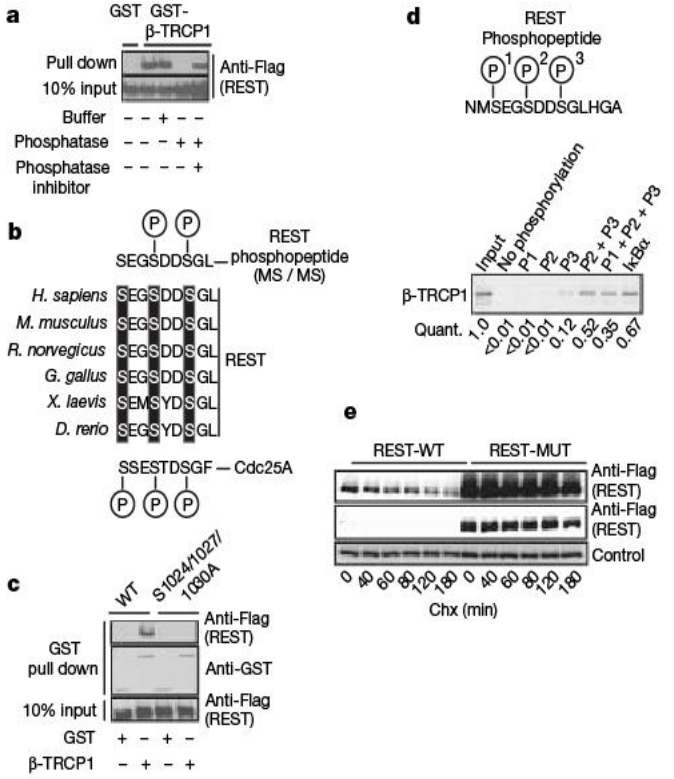

Figure 2 | A conserved phospho-degron in REST is required for regulation by β-TRCP. **a**, 293T cells were transfected with GST, GST-β-TRCP or Flag-REST expression plasmids. Flag-REST lysates were treated with buffer, λ-phosphatase or λ-phosphatase plus phosphatase inhibitor as indicated. Flag-REST lysates were then mixed with GST or GST-β-TRCP lysates, precipitated with glutathione beads and immunoblotted with anti-Flag antibodies. **b**, Phosphorylation of the conserved REST degron *in vivo*. Sequence alignments of REST proteins (*Hs* REST residues 1024–1032) from several species and the phospho-degron from *Hs* CDC25A. Phospho-serines within the REST degron identified by MS/MS are shown in upper sequence. **c**, 293T cells expressing the indicated combinations of GST, GST-β-TRCP1 (denoted on bottom) and Flag-REST mutants (denoted at top). GST-bound complexes were immunoblotted with anti-Flag (upper and lower panels) or anti-GST (middle panel). **d**, ^{35}S-β-TRCP1 was transcribed/translated *in vitro* and incubated with biotin-conjugated peptides spanning the REST degron (unphosphorylated or phosphorylated) or the IκB degron (phosphorylated). Peptide-associated proteins were precipitated with streptavidin-conjugated beads, analysed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE), and quantified using a phospho-imager. Peptide sequence spanning the REST degron is shown in the top panel. **e**, 293T cells expressing wild-type or degron-mutant Flag-REST complementary DNAs (cDNAs), were examined for Flag-REST protein half-life in a cycloheximide (Chx) timecourse.

human telomerase catalytic subunit (hTERT) and the SV40 LT oncogene ('TLM-HMECs'[16]) were transduced with a control or GFP-β-TRCP1-expressing retrovirus. Stable ectopic expression of β-TRCP1 resulted in reduced REST abundance (Fig. 3a) and robust anchorage-independent proliferation (Fig. 3b), thus phenocopying REST loss-of-function[4]. This is consistent with a transgenic mouse model in which ectopic β-TRCP1 expression in the mammary gland produced advanced breast cancer[17]. To determine whether REST degradation is critical for β-TRCP1-mediated transformation, TLM-HMECs stably expressing β-TRCP1 were transduced with retroviruses expressing wild-type or degron-mutant REST. Exogenous REST expression did not alter proliferation on an adhesive cell culture surface (Fig. 3c). In contrast, β-TRCP1-induced anchorage-independent proliferation was severely impaired by restoring REST expression (Fig. 3d). Consistent with its increased stability, degron-defective REST suppressed β-TRCP1-transformation more efficiently (Fig. 3d and Supplementary Fig. 8). These data implicate REST as an essential target in β-TRCP-driven oncogenic transformation.

Although REST is a well-documented regulator of neuronal gene expression and has been proposed to restrain several steps in neurogenesis (reviewed in ref. 3), its role in neurogenesis has not been tested genetically. Thus, we used embryonic stem cells to examine genetically the roles of REST and β-TRCP in the differentiation programme of neural stem and progenitor cells (reviewed in ref. 18). For this we employed embryonic stem cells in which eGFP was recombined into the Sox1 locus[19,20] ('46c cells'), a well-characterized marker of early neural differentiation *in vitro* and *in vivo*.

We first confirmed that endogenous REST stability is regulated during neural differentiation of 46c cells. As shown in Fig. 4a, REST half-life declined twofold in differentiated cells, consistent with the decreased REST stability observed in homogeneous neural stem cells (Fig. 1a). This decrease may be driven, in part, by a concomitant 13-fold increase in β-TRCP1 expression (Fig. 4b). To test the role of REST and β-TRCP in this differentiation programme, 46c cells were transfected with control, REST or β-TRCP1-targeting siRNAs alone or in combination, and subsequently cultured in differentiation

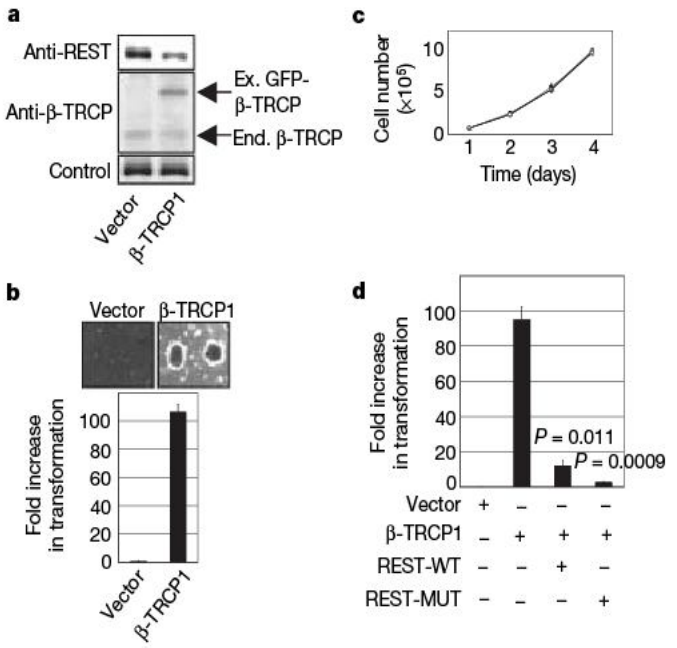

Figure 3 | β-TRCP targets REST during oncogenic transformation. **a**, TLM-HMECs were transduced with control or GFP-β-TRCP1-expressing retroviruses. Lysates were probed with antibodies against REST (upper panel), β-TRCP (middle panel) or vinculin (lower panel). **b**, Cells from **a** were analysed for anchorage-independent colony formation. Assays were performed in quadruplicate (error bars ± s.d.). Representative of three independent experiments is shown. **c**, HMECs were transduced with retroviruses expressing wild-type REST (REST-WT), degron-mutant REST (REST-MUT) and/or β-TRCP1. Cell numbers were monitored for four days after plating on tissue-culture dishes. Open circles, vector-1 + vector-2; filled circles, β-TRCP1; open triangles, β-TRCP1 + vector-2; filled diamonds, β-TRCP1+REST-WT; open squares, β-TRCP1 + REST-MUT. **d**, Cells from **c** were assessed for anchorage-independent colony formation. Assays were performed in triplicate (error bars ± s.d.). Representative of two independent experiments is shown.

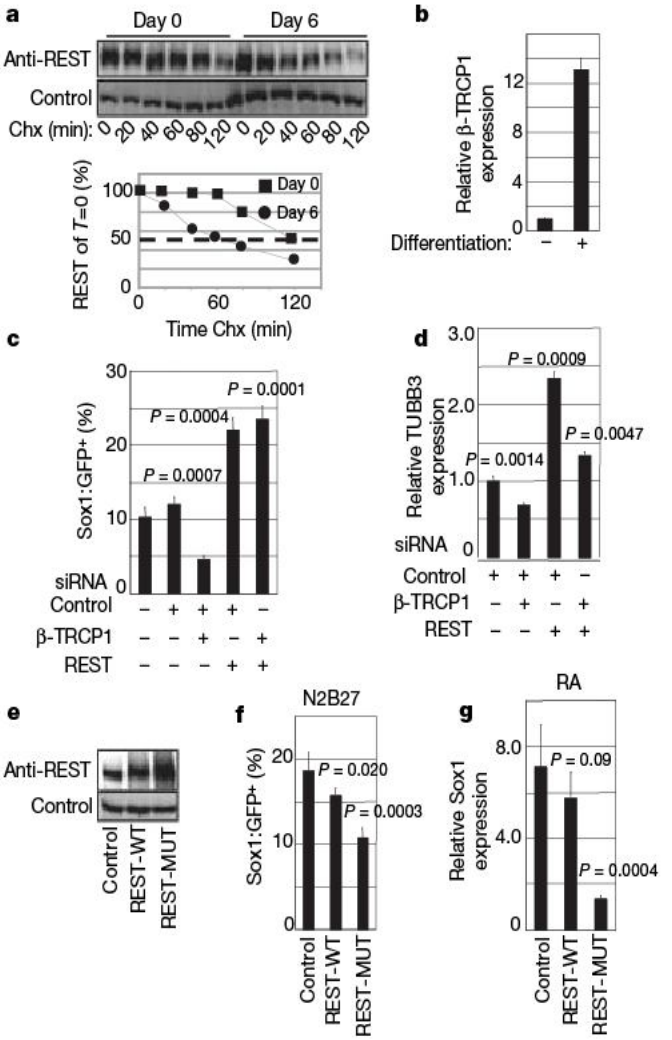

Figure 4 | The β-TRCP-REST pathway controls neural differentiation. **a**, Embryonic stem cells differentiated for 0 or 6 days were examined for REST protein half-life in a cycloheximide (Chx) time course. Differentiated lysates were analysed at three times the concentration of undifferentiated lysates. Quantification of REST levels is shown in the lower panel. **b**, Embryonic stem cells were differentiated for 0 or 6 days. β-TRCP1 mRNA was analysed by quantitative real-time PCR (qRT–PCR), and normalized to GAPDH mRNA abundance. Experiment was performed in triplicate (error bars ± s.d.). **c**, 46C cells transfected with the indicated combination of siRNAs were differentiated in N2B27 medium and analysed for Sox1:GFP expression by flow cytometry. Experiments were performed in quadruplicate (error bars ± s.d.) and are representative of four independent experiments. **d**, 46C cells from **c** were analysed for expression of TUBB3 mRNA by qRT–PCR, and normalized to GAPDH mRNA abundance. Experiments were performed in triplicate (error bars ± s.d.). **e**, 46C cells expressing control, Flag-REST-WT or Flag-REST-MUT (triple point mutation in the REST-degron) cDNA were immunoblotted with anti-REST (upper panel) or anti-vinculin (lower panel) antibodies. Note: in this experiment, exogenous REST was expressed at levels higher than endogenous REST. **f**, 46C cells from **e** were cultured in N2B27 differentiation medium and analysed for Sox1:GFP fluorescence. This experiment was performed in sextuplicate (error bars ± s.d.) and is representative of two independent experiments. **g**, Embryonic stem cells were infected as in **e**, differentiated into the neural lineage using an embryoid body–retinoic acid protocol, and analysed for Sox1 mRNA by qRT–PCR (normalized to GAPDH mRNA abundance). Experiment was performed in triplicate (error bars ± s.d.).

media and analysed for neural differentiation by flow cytometric analysis of Sox1:eGFP fluorescence. Inactivation of REST promoted differentiation, correlating with the efficiency of REST knockdown (Fig. 4c and Supplementary Fig. 10a, b), thus providing the first genetic evidence that REST negatively regulates early neural differentiation. Conversely, siRNAs that suppress β-TRCP1 expression more than 90% (Supplementary Fig. 11c) attenuate differentiation into the neural lineage (Fig. 4c). These results were confirmed in multiple time points (data not shown) and with multiple siRNAs (Supplementary Fig. 10b, d). Importantly, simultaneous REST+β-TRCP1 knockdown increased Sox1:eGFP-positive cells more than fivefold relative to β-TRCP1-siRNA alone (Fig. 4c), showing REST reduction restores neural differentiation in the absence of β-TRCP. Similar results were observed by measuring the abundance of an independent neuronal marker, TUBB3 (Fig. 4d). Thus, downregulation of REST is a critical function of β-TRCP during early neural differentiation.

These data support the model that β-TRCP regulates neural differentiation by facilitating REST degradation and predicts that a non-degradable REST would impede neural differentiation. To test this, we first examined the stability of wild-type or degron-mutant REST expressed in the context of neural differentiation. In this experiment, REST transgenes were expressed at levels much lower than endogenous REST to prevent alterations in differentiation kinetics (see below). Notably, the stability of endogenous REST and wild-type exogenous REST decreased similarly during neural differentiation (Supplementary Fig. 10e and Fig. 4a). In contrast, degron-mutant REST was stable, regardless of the cellular differentiation status (Supplementary Fig. 10e). To test whether REST stabilization alters neural differentiation, 46c cells were transduced with high-titre retroviruses expressing wild-type or degron-mutant REST, resulting in a 1.5- and 2.6-fold increase in total REST (Fig. 4e). Notably, both transgenes attenuated neural differentiation, with the degron-mutant REST eliciting a more dramatic phenotype (Fig. 4f).

To demonstrate REST's role in neural differentiation further, we used an independent neural differentiation assay. Embryonic stem cells stably expressing wild-type or degron-mutant REST were differentiated by formation of embryoid bodies followed by stimulation with retinoic acid, a protocol routinely used to differentiate embryonic stem cells into the neuronal lineage[21]. In this context, non-degradable REST suppressed differentiation more than fivefold as measured by messenger RNA (mRNA) expression of Sox1 (Fig. 4g). Collectively, these observations strongly link β-TRCP function and REST degradation in controlling neural differentiation.

Here we demonstrate that REST is a labile protein targeted for ubiquitin-dependent proteasomal degradation by $SCF^{\beta\text{-}TRCP}$ through a phospho-degron on REST. We show $SCF^{\beta\text{-}TRCP}$ is a critical regulator of both physiological and pathological REST activities, constituting a new pathway controlling neural differentiation and cellular transformation (see Supplementary Fig. 11). We provide the first genetic evidence that REST and $SCF^{\beta\text{-}TRCP}$ regulate an early stage in neural specification. Our data are consistent with a model in which developmental cues induce degradation of REST, resulting in the de-repression of proneural REST targets. The ability of REST to inhibit terminal differentiation of neurons also predicts that it may promote proliferative properties in the neuronal lineage when overproduced or inappropriately stabilized. Consistent with this notion, REST is overexpressed in human medulloblastoma, and ectopic REST expression in *v-myc*-immortalized neural stem cells promotes medulloblastoma formation in mice[22,23]. Thus, the contrasting roles of REST as an oncogene and tumour suppressor are highly dependent on the developmental lineages.

β-TRCP is overexpressed and oncogenic in epithelial cancers[17,24,25], and we identified REST as a key target in this context. This suggests that pharmacological inhibition of β-TRCP may provide a way of restoring REST tumour suppressor function in human cancer. The presence of a phospho-degron motif within REST suggests a role for upstream kinase(s) and/or phosphatase(s) that control REST degradation. We propose a model in which differentiation into the neural state is induced by this yet to be discovered signal transduction cascade that targets REST for degradation by $SCF^{\beta\text{-}TRCP}$, acting cooperatively with induction of β-TRCP expression during neural differentiation. Conversely, hyperactivation of such pathway(s) priming REST degradation may be oncogenic in epithelial tissues and thus serve as new therapeutic targets in cancers with compromised REST function. Thus, exploration of these pathways will probably provide new opportunities for modulating neural stem-cell and cancer-cell behaviour.

METHODS SUMMARY

siRNA screen. For the initial F-box siRNA screen, 272 siRNAs targeting 68 unique F-box proteins[26] (four siRNAs per F-box gene) were arrayed individually in 96-well format. 293T cells were transfected with 100 nM siRNAs using oligofectamine under standard conditions. On the subsequent day, cells were transfected with CMV-mRFP-REST plasmid DNA using mirus Trans-IT 293 under the manufacturer's recommended conditions. Cells were analysed for mRFP fluorescence after 48 h by flow cytometry. Subsequent testing of all candidate siRNAs was performed in triplicate and normalized to effects on mRFP (two independent experiments).

Neural differentiation. 46C embryonic stem cells were induced to differentiate by culture in serum-free N2B27 medium (1:1 ratio of DMEM/F12 and Neurobasal medium, supplemented with N2 and B27 (Invitrogen)). For embryoid body formation, cells were plated in Corning ultra-low attachment six-well plates at 5×10^5 cells per well in embryonic stem medium without LIF. Medium was changed every two days, and retinoic acid was added from day 4 to day 8 to 1 μM final concentration.

Transformation assays. Retroviral infections of HMECs were performed with indicated viral supernatants in the presence of 8 $\mu g\,ml^{-1}$ polybrene, and transduced cells were selected for resistance to the appropriate drug: puromycin (2.0 $\mu g\,ml^{-1}$), neomycin (200 $\mu g\,ml^{-1}$). Anchorage-independent proliferation assays were performed as previously described[4]. For each assay, the average of at least three replicates ± s.d. is shown.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 10 September 2007; accepted 22 January 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank G. Mandel and R. Truant for providing REST-C antibodies and mRFP-REST cDNA, respectively. We thank J. Luo, D. Nguyen and D. Lee for suggestions and reading of the manuscript. We are grateful to R. Mulligan, A. Balazs, J. Jin, M. Sheng and V. Dixit for providing reagents, and to D. Guardavaccaro and M. Pagano for communicating results before publication. T.F.W. is a fellow of the Susan G. Komen for the Cure. G.H. is a Helen Hay Whitney post-doctoral fellow. X.L.A. is supported by a National Institutes of Health predoctoral fellowship. This work was supported by National Institutes of Health grants to Y.S., J.W.H., and a US Army Innovator Award to S.J.E. S.J.E. is an Investigator with the Howard Hughes Medical Institute.

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LETTERS

X-ray structure of a prokaryotic pentameric ligand-gated ion channel

Ricarda J. C. Hilf[1] & Raimund Dutzler[1]

Pentameric ligand-gated ion channels (pLGICs) are key players in the early events of electrical signal transduction at chemical synapses. The family codes for a structurally conserved scaffold of channel proteins that open in response to the binding of neurotransmitter molecules. All proteins share a pentameric organization of identical or related subunits that consist of an extracellular ligand-binding domain followed by a transmembrane channel domain. The nicotinic acetylcholine receptor (nAChR) is the most thoroughly studied member of the pLGIC family (for recent reviews see refs 1–3). Two sources of structural information provided an architectural framework for the family. The structure of the soluble acetylcholine-binding protein (AChBP) defined the organization of the extracellular domain and revealed the chemical basis of ligand interaction[4–6]. Electron microscopy studies of the nAChR from *Torpedo* electric ray have yielded a picture of the full-length protein and have recently led to the interpretation of an electron density map at 4.0 Å resolution[7–9]. Despite the wealth of experimental information, high-resolution structures of any family member have so far not been available. Until recently, the pLGICs were believed to be only expressed in multicellular eukaryotic organisms. The abundance of prokaryotic genome sequences, however, allowed the identification of several homologous proteins in bacterial sources[10,11]. Here we present the X-ray structure of a prokaryotic pLGIC from the bacterium *Erwinia chrysanthemi* (ELIC) at 3.3 Å resolution. Our study reveals the first structure of a pLGIC at high resolution and provides an important model system for the investigation of the general mechanisms of ion permeation and gating within the family.

ELIC is similar in length and sequence to its orthologue from the cyanobacterium *Gloebacter violaceous* (sharing 18% of identical amino acids) and it shows considerable homology to eukaryotic family members (with 16% sequence identity to nAChRα, Supplementary Fig. 1). When investigated in artificial lipid bilayers, the protein mediates cation-selective currents but it does not discriminate between different monovalent cations such as Na^+, K^+ and Cs^+, a functional behaviour that closely resembles the selectivity of acetylcholine and serotonin receptors[12] (Supplementary Fig. 2). The crystal structure of ELIC has been determined at 3.3 Å resolution by the SIRAS (single isomorphous replacement anomalous scattering) method (Supplementary Table 1, and Supplementary Figs 3 and 4). The pentameric protein is shown in Fig. 1. The five subunits are arranged like the staves of a barrel around a symmetry axis that defines the ion permeation path. The overall dimensions of the protein (95 Å × 110 Å) closely resemble the acetylcholine receptor ion channel, not including the cytoplasmic region, which is absent in ELIC. The subunits are tightly interacting in both, their extracellular and their membrane embedded part with adjacent subunits burying more than 5,000 $Å^2$ of the combined molecular surface. On the extracellular side, the protein subunits enclose a wide, aqueous, cylindrical vestibule with a diameter of about 16 Å that is lined by charged and hydrophilic residues. This vestibule narrows down at the membrane interface to a discontinuous, partly hydrophobic pore with a maximum diameter of about 7 Å that probably defines a closed conformation of the channel.

Each protein subunit is organized in two halves: the amino-terminal half that constitutes the extracellular domain and a carboxy-terminal half that makes up the pore domain (Fig. 1c). Both termini of the protein chain are located on the extracellular side. The extracellular domain consists of ten β-strands that are organized in two sheets to form a β-sandwich and a short α-helix. The topological organization of the extracellular domain of ELIC is very similar to its eukaryotic counterparts and to AChBP, except for an N-terminal α-helix that is abundant in eukaryotic proteins and missing in bacterial pLGICs. The regions of conserved sequence include the central part of the 'Cys-loop' that connects β6 and β7 but does not extend to the flanking disulphide-bridged cysteinse residues that are strictly conserved among eukaryotic pLGICs (Supplementary Fig. 1a). The transmembrane region consists of four α-helices that are connected by short loops. These four helices (named α1–α4) are equivalent to the previously described transmembrane regions M1–M4 of the acetylcholine receptor[8]. Three helices (α1–α3) span the membrane with small tilts with respect to its plane and form a tightly interacting bundle. In the pentameric protein, these helices are arranged in two concentric layers around the pore axis: an inner circle that is formed by helix α2 and an outer circle defined by the helices α1 and α3. Only residues of α2 contribute to the pore lining, whereas α1 and α3 appear to shield and stabilize the pore. All three segments are involved in interactions at subunit interfaces, whereas the fourth helix, α4, is located at the periphery of this barrel-like arrangement; it only loosely interacts with α1 and α3 and is not involved in subunit–subunit interactions (Fig. 1a). In an attempt to study the interaction of monovalent cations with ELIC, we soaked our crystals in solutions that only contain the respective cation, and we identified the bound ions by anomalous and isomorphous difference Fourier techniques (Supplementary Table 2). In that way, we studied the binding of Rb^+, Cs^+ and Tl^+ ions to the protein, which have been shown to permeate through ELIC and the related nAChR channels[12] (Supplementary Fig. 2c, d). Although this approach did not allow the identification of specific binding sites in the pore region, it revealed ordered binding of ions to sites in the extracellular ligand-binding domain (Supplementary Fig. 5).

The conservation in the extracellular part of ELIC becomes evident when comparing its structure with the structure of the homopentameric AChBP, which has proved to be a valuable representative for the ligand-binding domain[4]. A superposition of the two domains is shown in Fig. 2a. Three hundred and fifty Cα positions at the N-terminal half of the ligand-binding domain of the five subunits superimpose with a root mean square deviation of less than 1.5 Å. The largest differences are found in loop regions that interact with the transmembrane channel

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and which are naturally absent in the soluble binding protein. The ligand-binding site of AChBP that is also conserved among acetylcholine receptors is located in a pocket of the protein at the interface between two adjacent subunits (Fig. 2a). This pocket is covered on one side by an extended loop region connecting β9 and β10. In ELIC, the tip of this loop is mobile and thus only weakly defined in its electron density, which is consistent with the increased mobility of this region that has been reported for AChBP in the absence of bound ligand[6]. Although the ligands that trigger pore opening in ELIC have not yet been identified, it is interesting to compare the structures of the equivalent regions. The acetylcholine-binding site contains conserved aromatic amino acids from dispersed regions of the protein, which contribute to an aromatic-binding pocket for quaternary ammonium compounds that are stabilized by so-called 'cation–π' interactions[5,13] (Supplementary Fig. 6a). Although the ligand-promoting channel opening in ELIC could be as small as a proton, as has been suggested for the homologous GIvi channel[11], it is remarkable to find several of these aromatic residues conserved in the ELIC structure (Fig. 2b and Supplementary Fig. 6b). Ordered binding of Cs^+ and Tl^+ ions emphasizes the electrostatic attraction for cations in this region and underscores its role as a potential ligand-binding site that could promote channel opening like in eukaryotic pLGICs.

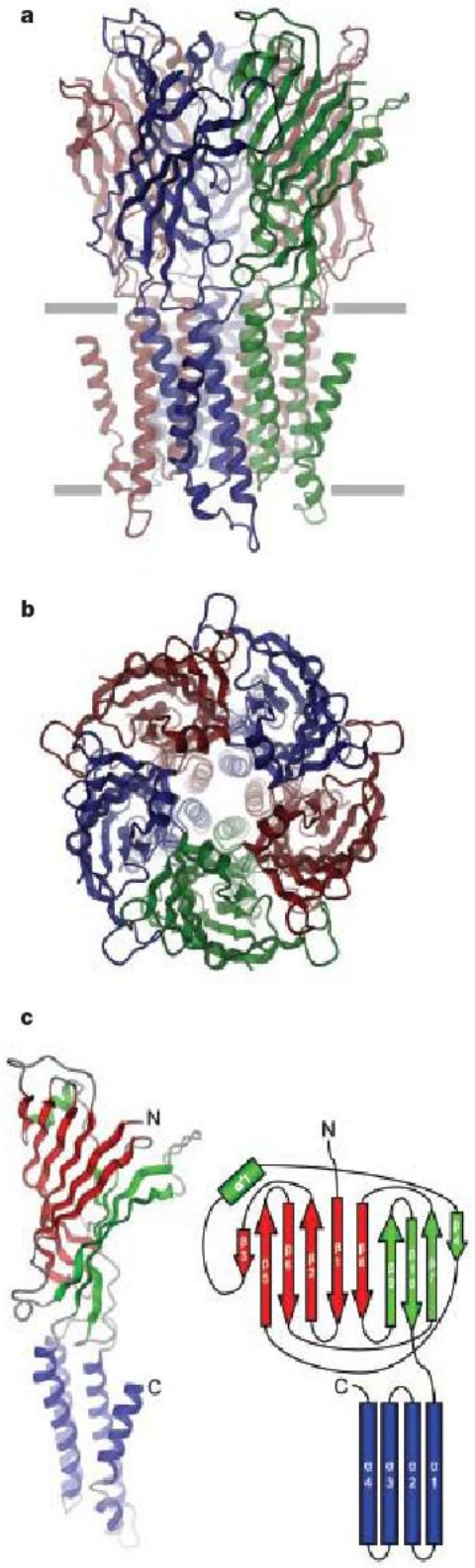

Figure 1 | ELIC structure. **a**, Ribbon representation of ELIC viewed from within the membrane with the extracellular solution above. The approximate membrane boundaries are indicated. **b**, Structure of the pentameric channel viewed from the extracellular side. **c**, Structure and topology of the ELIC subunit. The subunit is viewed from within the membrane. Secondary structure elements constituting the two sheets of the β-sandwich of the extracellular domain are coloured in red and green, respectively. The four helices of the pore region are coloured in blue. Figures 1–3 were prepared with DINO (http://www.dino3d.org).

Ligand-dependent gating involves the transduction of conformational rearrangements from the ligand-binding domain to the pore[2,14]. This process is mediated through interfacial contacts between the two domains[9]. The interface between the extracellular and pore domains is well defined in the ELIC structure and it involves, next to the covalent connection between β10 and α1, residues of three loop regions in the extracellular domain that contact the α2–α3 loop of the pore domain (Fig. 2c and Supplementary Fig. 6c). These interactions include conserved residues of the β6–β7 loop (the 'Cys' loop) and contacts to the β1–β2 turn and the β8–β9 loop of the neighbouring subunit. Several of those regions have previously been identified as influencing gating in different family members[2,15–17].

The wide and hydrophilic extracellular vestibule of ELIC leads into a narrow pore at the membrane boundary. The lumen of this pore is lined by residues of helix α2 that spans the membrane in a nearly perpendicular orientation. On its extracellular half, the pore is interrupted by bulky side chains that occlude a hydrophobic cavity that extends towards the centre of the membrane (Fig. 3a). This cavity is confined by Phe 246 on the extracellular side and by Leu 239 towards the cytoplasm. Below the constriction, a hydrophilic channel of width 6 Å leads from the membrane centre to the intracellular exit. The bulky hydrophobic side chains of Phe 246 and Leu 239 would prevent the diffusion of ions and thus probably serve as physical gates in the closed conformation of the channel. Unlike other ion-channel proteins that specifically bind the transported ions in narrow selectivity filters[18,19], we did not identify ordered binding of permeant ions in the pore of ELIC. However, we did observe binding of apolar Xe atoms in the hydrophobic cavity and at the extracellular side of Phe 246, thus underlining the hydrophobic nature of this region (Fig. 3a). When comparing the pore of ELIC with the structure of the equivalent region of nAChR, interesting differences become apparent. Unlike our structure, nAChR shows a continuous channel with a diameter of about 6 Å (ref. 9) (Fig. 3b and Supplementary Fig. 7). The difference in the pore size is most pronounced at the extracellular entry where the helices of nAChR are bent away from the channel axis, thus providing an aqueous funnel-shaped path in a region where the ELIC pore is occluded. On the intracellular side, the pore radius in both proteins is similar. This observation is in contrast to electrophysiological experiments on nAChR, which predicted a constriction towards the cytoplasm[20,21]. Apart from differences in the diameter, other features of the pore, such as the predominantly hydrophobic region at the membrane centre and the presence of acidic residues on both sides of the membrane boundary appear preserved.

An investigation of the electrostatic potential along the ion permeation path of ELIC reveals insight into the cation selectivity of the channel (Fig. 3b). The predominant negative potential throughout the channel results from the excess of acidic residues in the protein, which provide an attractive environment for cations despite the

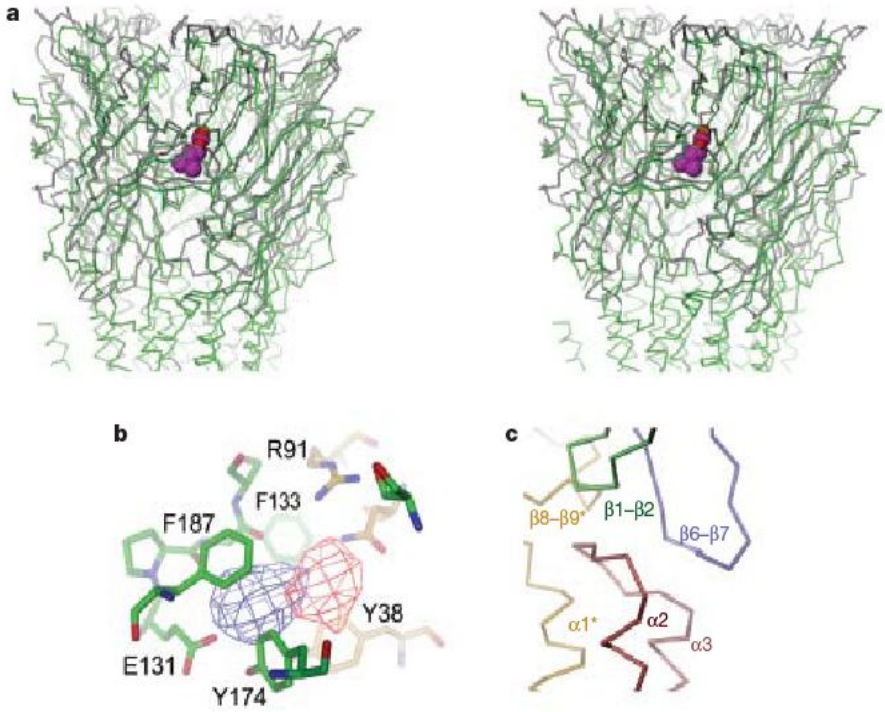

Figure 2 | Structure of the extracellular domain. **a**, Stereo view of AChBP (PDB code 1UV6) superimposed on the extracellular domain of ELIC. Proteins are shown as Cα representation and coloured in grey (AChBP) and green (ELIC). Carbamylcholine that is specifically bound to the acetylcholine-binding site of AChBP is shown as spacefilling model (carbon atoms in magenta). **b**, View of the putative ligand-binding site in ELIC. Carbon atoms of residues from the two subunits are coloured in green and orange, respectively. Selected residues are labelled. Anomalous difference electron density of bound Tl^+ and Cs^+ ions is contoured at 4σ and shown in blue and red, respectively. **c**, Cα representation of the interface region between the extracellular domain and the pore. The different elements are shown in unique colours, the 'Cys loop' (β6–β7) is coloured in blue. Residues of the neighbouring subunit are coloured in orange.

presence of positively charged residues in the extracellular vestibule, some of which are found close to the channel entrance. The electrostatic potential in the narrow pore region is dominated by acidic residues at the membrane boundary (for example, Glu 229 at the intracellular entrance). Because of the long-range nature of coulombic interactions and the low-dielectric environment of the membrane, the

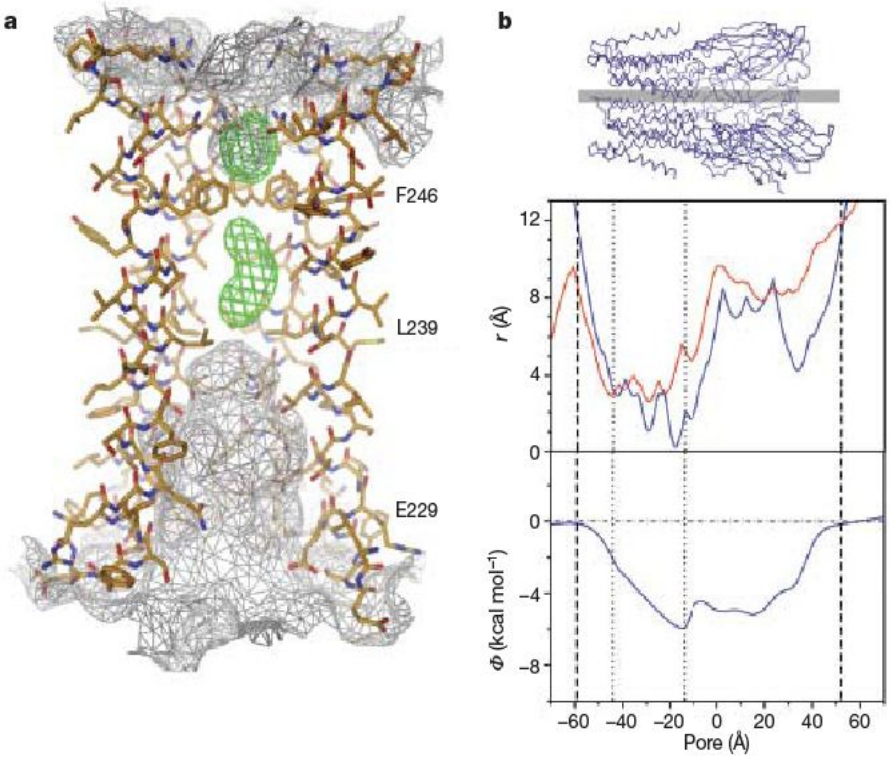

Figure 3 | ELIC pore. **a**, View of the α2 helices defining the pore region. The front subunit is removed for clarity. The molecular surface is shown as white mesh. Anomalous difference density of Xe atoms is contoured at 4σ and shown as green mesh. The positions of selected residues are indicated. **b**, Pore radius and electrostatic potential in the ELIC pore. Orientation of the ELIC channel is shown above. Molecular boundaries (dashed line) and transmembrane region (dotted line) are indicated. Top: pore radius of ELIC (blue) and nAChR (red) are shown. Bottom: electrostatic potential along the pore axis of ELIC as calculated from a numerical solution of the linearized Poisson–Boltzmann equation.

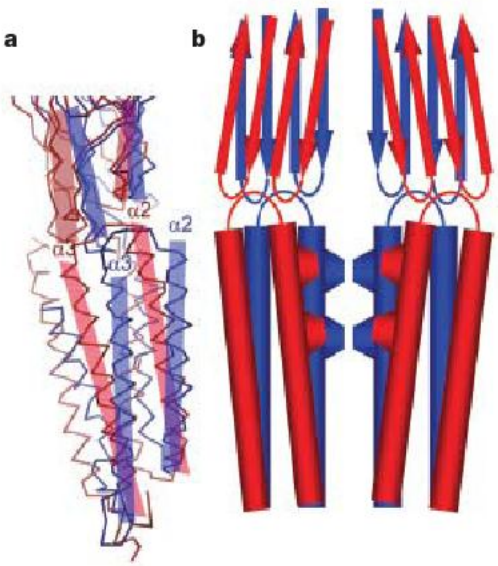

Figure 4 | Schematic model of pore opening. **a**, Superposition of selected regions from a single subunit of ELIC (blue) and the α-subunit of nAChR (red) close to the pore region. Differences in the helix tilt and the conformation of contacting groups from the extracellular region are highlighted. **b**, Schematic representation of two subunits, indicating a possible pore opening mechanism by a tilt of the helix at the extracellular side away from the pore axis in response to changes in the extracellular domain.

electrostatic potential in the channel is negative even though hydrophobic residues line the central pore region. This mechanism of electrostatic screening does not require the strict conservation of amino acids and may account for the fact that, although the pore regions of different family members are only weakly conserved, there is a common overall organization of hydrophobic residues in the centre and of charged residues at the periphery of the channel[22]. These charged residues have indeed been shown to confer ion selectivity to nAChR and other members of the family[23–27]. Although such a channel architecture allows for a discrimination of ions based on their charge, it does not allow for exquisite selectivity between two similar ions as found in K^+ channels[18].

Large hydrophobic residues in the centre of the pore have previously been shown to play an important role in channel closing in nAChR[8,20,28]. It has been proposed that these hydrophobic residues would impede ion permeation by a process called 'hydrophobic gating' as long as the channel does not exceed a certain size[29]. Unlike in the structure of nAChR, which shows a narrow but continuous pore, in ELIC these residues physically obstruct the channel. It will thus be interesting to see the conformational rearrangements leading to channel opening.

Different mechanisms of gating of pLGICs have been proposed in previous studies[8,21,30]. Although we do not want to engage in speculation about gating in the absence of additional data, it is interesting to compare the different conformations of the pore helices in ELIC and nAChR (Fig. 4). Both structures are believed to represent closed conformations of the respective proteins. If, however, the nAChR structure were to show a conformation that is closer to the structure of a conducting state, the difference might hint at a possible opening mechanism by an outward tilt of the pore helices on the extracellular side away from the channel axis (Fig. 4). This movement would be triggered by ligand binding to the extracellular domain and would be transmitted through the domain–domain interface.

The structure of ELIC shows the pore of a pLGIC in a non-conducting conformation. The protein shares many conserved features with its eukaryotic counterparts, which suggests that the basic mechanisms of ion permeation and gating are preserved across the prokaryotic–eukaryotic species boundary. Future studies will have to identify the ligands that promote channel opening and show the structure of a conducting conformation.

METHODS SUMMARY

ELIC was expressed in *Escherichia coli* with its N terminus fused to maltose-binding protein (MBP) preceded by a signal sequence (PelB). The protein was purified from isolated membranes in the detergent *n*-undecyl-β-D-maltoside. MBP was cleaved during purification by proteolytic digestion at a specific protease cleavage site located between MBP and the channel protein. Crystals were grown at pH 6.5 with addition of 13% PEG 4000, 200 mM ammonium sulphate and 0.5 mg ml^{-1} *E. coli* lipids. Data were collected at the X06-SA beamline at the Swiss Light Source of the Paul Scherrer Institute on a Pilatus detector (Dectris) (Supplementary Tables 1 and 2). The crystals were of space group $P2_1$ with two homo-pentameric channels in the asymmetric unit (Supplementary Fig. 4). The structure was determined by the SIRAS method, and phases were extended by cyclic tenfold non-crystallographic symmetry (NCS) averaging (Supplementary Fig. 3). All subunits were structurally very similar and were refined by maintaining strong constraints throughout (Supplementary Table 1). The final structure encompassing residues 11–316 was well refined, with good stereochemistry and no outliers in disallowed regions of the Ramachandran plot. For electrophysiological characterization in artificial lipid bilayers, the protein was reconstituted into liposomes and studied in a bilayer system.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 17 October 2007; accepted 15 January 2008.
Published online 5 March 2008.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank I. Toth from the Scottish Crop Research Institute for providing g-DNA of *E. chrysanthemi*, B. Blattmann and A. Haisch for assistance with crystal screening, D. Sargent for help with Xe derivatization, C. Schulze-Briese and the staff of the X06SA beamline for support during data collection, the Protein Analysis Group at the Functional Genomics Center of the University of Zürich for help with mass spectrometry, R. MacKinnon for comments on the manuscript and members of the Dutzler laboratory for help in all stages of the project. Data collection was performed at the Swiss Light Source of the Paul Scherrer Institute. This work was supported by a grant from the National Center for Competence in Research in Structural Biology and the EMBO Young Investigator Program to R.D. R.J.C.H. is affiliated with the Molecular Life Sciences Ph.D. Program of the University/ETH Zürich.

Author Contributions R.D. and R.J.C.H. designed the project. R.J.C.H. performed all experiments. R.D. assisted in data collection, structure determination and electrostatic calculations. R.D. and R.J.C.H. jointly wrote the manuscript.

Author Information Coordinates have been deposited in the Protein Data Bank under code 2vl0. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.D. (dutzler@bioc.uzh.ch).

CORRIGENDUM

doi:10.1038/nature06845

Structural basis for the function and inhibition of an influenza virus proton channel

Amanda L. Stouffer, Rudresh Acharya, David Salom, Anna S. Levine, Luigi Di Costanzo, Cinque S. Soto, Valentina Tereshko, Vikas Nanda, Steven Stayrook & William F. DeGrado

Nature 451, 596–599 (2008)

In this Letter, the Protein Data Bank (PDB ID) code for the amantadine complex structure was inadvertently omitted; it should be 3C9J.

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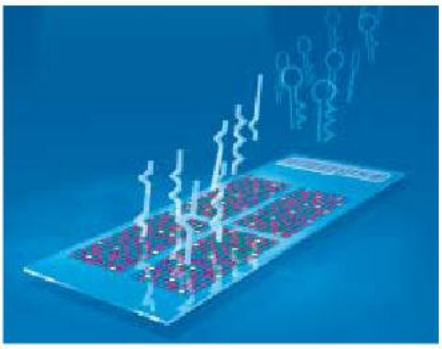

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Miltenyi Biotec announces the new microRNA-specific **miRXplore Microarray Kits** and **Services**. The microarray contains more than 2700 mammalian and viral microRNAs as deposited in the miRBase database (version 10.1). Developed and validated in close co-operation with leading microRNA experts at Rockefeller University, a specific and sensitive microRNA signal detection is possible down to 0.01 fmol. Furthermore, the microarray's extensive control system contains multiple positive, negative, and calibration controls for sample-independent normalization as well as monitoring of critical experimental steps. Two service options are available; one of them is the miRXplore Microarray Universal Reference Service. Here, a unique synthetic microRNA pool, containing more than 1000 microRNAs in equimolar concentration, is used as a reference, hybridization control, or as a quality control for miRNA microarray hybridizations. Plus, when co-hybridized with each sample, it enables a calculated indirect comparison of sample collections—ultimately saving sample material.

NanoPrint Microarrayers, from **ArrayIt**, are industrial-level platforms for all microarray manufacturing applications including DNA microarrays, protein microarrays, and other types of biomolecules. NanoPrint systems enable the manufacture of microarrays utilizing ArrayIt Professional, 946 and Stealth Style Micro Spotting Pins. The NanoPrint uses superior linear drive motion control technology and proprietary Warp1 controllers, and is compatible with all standard microarray surfaces made by ArrayIt and other open platform glass substrate slide vendors. Systems are easily configured to print onto different types of surfaces including 96-well plates, plate-sized glass, and proprietary cartridges and cassettes by taking advantage of flexible and modular deck configurations and an easy to use software interface. The Microarray Manager Software combines unparalleled power and simplicity in a graphical user interface.

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As the funding woes of scientists trying to secure a grant from the US National Institutes of Health (NIH) continue (see *Nature* **452,** 249; 2008), one can't help but wonder: should the wealth be spread more evenly? A News story on page 258 explores this possibility — and notes some of the more eye-popping grant numbers of a select few 'grandee grantees'. Many bright, well-educated, qualified scientists struggle to get their first big NIH grant, yet last year, 200 more established researchers were supported by six or more grants each. One principal investigator earned 32 grants; many had eight or nine.

There's an important caveat here. Some multiple grant-getters seem to be doing well because they organize science conferences or run training workshops, where each event is funded by a separate smaller grant, inflating the numbers. But plenty of principal investigators do seem to have the knack — and the drive and the means — for collecting multiple research grants.

When you hear stories about exceptional young scientists who are struggling to sustain their labs, this situation seems a little unfair. Jill Rafael-Fortney, for example, is working on muscular dystrophy at Ohio State University in Columbus. She says that she had to downsize her lab and let go of experienced postdocs because she had only limited funds. She is one of 12 young biomedical scientists featured in *A Broken Pipeline?* — a report published last week by a group of US research institutions and universities (see www.brokenpipeline.org).

Looking to spread the wealth a bit more, NIH director Elias Zerhouni is considering requiring that grantees to spend at least 20% of their time on each of the grants they are awarded. Yet there's no reason why the more productive labs should be punished. If the system becomes need- rather than merit-based, the best science may not get the support it deserves. Unfortunately this won't comfort the enterprising biomedical researcher who has several high-profile publications, cutting-edge ideas, and a long-time dream of earning the support of the nation's leading biomedical institution —and is still waiting.

Gene Russo is editor of *Naturejobs*.

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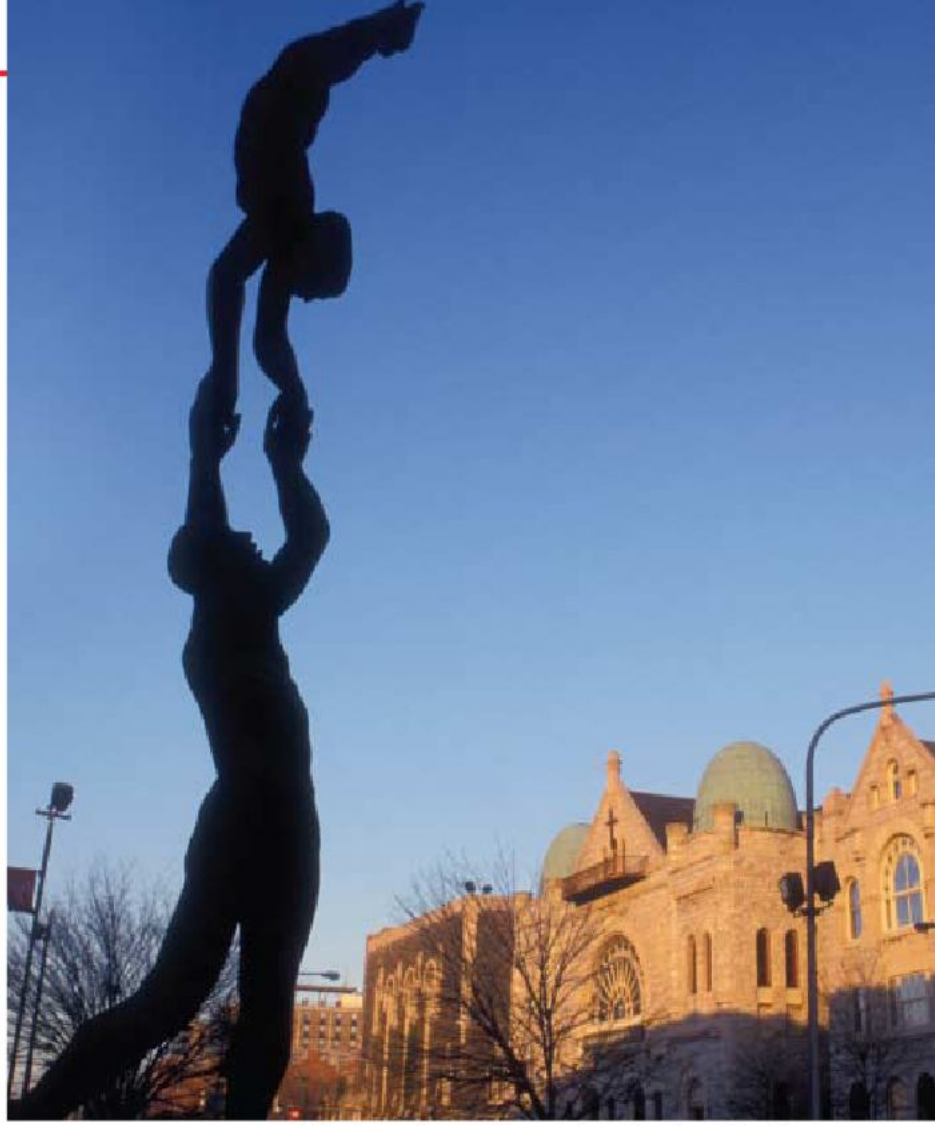
Closing the gaps

US researchers are keen to find ways to address health disparities among minorities. **Paul Smaglik** reports.



NEWSCOM

It took Ala Stanford Frey 18 years of training to become a paediatric surgeon. It took a month of violence to get her involved in examining why ethnic minorities have more diseases and injuries than the majority population. Frey, one of only two female African American paediatric surgeons in the United States, was raised by a single mother in impoverished and crime-ridden North Philadelphia, Pennsylvania. She returned to Philadelphia in 2006, wanting to give something back to the community. She thought her contributions would come through surgery. Then the shootings escalated.

"So many people were being shot — two to three a day in September 2006," she says. Some would return for follow-ups after surgery; many wouldn't, because they lacked health insurance or feared questioning by police. Those fears jeopardized the patients' long-term health, Frey says. She expressed her concerns to the dean of the medical school at Temple University, where she worked, and was told that such occurrences were part of a bigger picture. "He said this was a health-disparity issue," Frey recalls.

Dramatic example

A disproportionate rate of gunshot injuries in a minority community is a dramatic example of public-health disparity, and it isn't the only one. Latinos and African Americans together make up just over a quarter of the US population of 300 million, but diabetes and some forms of cancer and heart disease strike these groups almost twice as much as the general population.

Many minority researchers want to investigate health problems facing their own communities. Funding from the National Center on Minority Health and Health Disparities in Bethesda, Maryland, is helping them establish centres of excellence and research collaborations. Scientists who follow these paths are finding rich multidisciplinary opportunities, including researching genetic and molecular differences among races, conducting clinical trials that capture under-represented groups, and studying aspects of behaviour and environment affecting minority health.

Bernard Miller, a researcher at Washington University in St Louis, Missouri, and a participant in the National Institutes of Health (NIH) minority network, is looking at the roles of both behaviour and biology in diabetes. When Miller went to Washington University as a fellow, he worked with Sam Klein, who was studying triglycerides and fatty acids. Miller noticed "black and white differences" — literally — in metabolism. African Americans in the study had more difficulty in clearing fatty acids and triglycerides. Those differences could lead to obesity and diabetes.

Focusing on minorities: Ala Stanford Frey (top) and Maria Rosario Araneta.

Miller says that, overall, there is ample funding for diabetes research in the United States. But he adds that the NIH often directs money towards molecular mechanisms, when it should focus on ways to combat obesity and investigate metabolism — issues that seem specific to minority populations. Miller is comparing majority and minority groups with diabetes and seeing how both react to medical and lifestyle interventions.

Maria Rosario Araneta, a diabetes researcher at the University of San Diego, California, is also investigating the differences in diabetes, but among people of her own Filipino ethnicity. Their rate of type-2 diabetes is even higher than among African Americans — even in populations not considered obese. "Biologically, we're very different," she says. Comparing a study on Filipinos with the results of an earlier, broader longitudinal study on diet and health, she noticed that type-2 diabetes occurred four times more often in normal-weight Filipinos compared with Caucasians. At a conference, Araneta heard about a group of relatively thin diabetic Filipino men at a US veteran hospital. "It was baffling, because most of the other diabetes patients were obese," says Araneta. It is stranger still, she says, given that they have access to care — a peculiarity that deserves further investigation. Many health researchers cite lack of access to health care as a major factor in disease disparities.

Although Araneta receives funding from the NIH, she's had difficulty obtaining grants to investigate the Filipino population. One grant response called the Filipino cohort "a low-impact population" and added that the same health information could be obtained from studying the Japanese population. Araneta says

Temple University: working on health issues for minority groups.

TEMPLE UNIV.

the two groups have enough cultural and genetic differences to warrant separate study.

Apart from differences in disease prevalence, minority groups sometimes disproportionately experience complications. Leonor Nunez, a researcher at Duke University Medical Center in Durham, North Carolina, says that some researchers, minority and otherwise, are finding genetic markers that provide clues to differences in disease pathology. She has noticed that Hispanic people with diabetes have a higher incidence of retinopathy and wonders whether there is a biological or genetic basis for this.

Filling the ranks

Enrolling minority patients in clinical trials can be difficult because some groups distrust the US medical community. Some Latinos worry that their immigration status will be questioned; many African Americans remember the 40-year Tuskegee experiment, in which doctors withheld treatment from African American men with syphilis to study how the disease progressed.

Having minority scientists involved in clinical trials can help. But there are not enough to go around. Filipinos are roughly as likely as the average population to be doctors; Latinos and African Americans much less so. Historically black colleges and universities, founded before desegregation laws that came into effect in 1964, are attempting to address that. Sandra Harris-Hooker, director of the minority biomedical research support programme at Morehouse School of Medicine in Atlanta, Georgia, says that Morehouse focuses on training physicians in health-disparities issues and so far doesn't have enough of its own researchers to build a strong research programme. To combat that, Morehouse is establishing ties to other universities.

"Because we're small, we do better if we collaborate both internally and externally," Harris-Hooker says. Collaborations have included neuroscience and cardiovascular research with Emory University and Georgia Institute of Technology both in Atlanta, Georgia, and work on cancer with the University of Alabama in Birmingham. Still, with less than 1% of the NIH's budget devoted to health disparities (see 'Slow progress on research funding'), these aren't enough to close the gap between minority groups and the general population, she says.

Jada Bussey-Jones, an assistant professor of medicine at Emory University, believes the patient–doctor relationship is part of the problem. She is researching minority perceptions of physicians and is writing curricula to help train doctors working with minorities. Perception needs to shift on both sides, she says. "No one wants to be called a racist, but experience might colour the lens in which we see the world or a patient."

George Littleton, associate dean of the graduate school at Howard University in Washington DC, says that minority researchers are making a difference by asking questions about health disparities that others might not ask. But differences in rates of high blood pressure, obesity, diabetes and prostate cancer are too prevalent to be addressed by minority scientists alone — even though historically black colleges and universities, such as Howard, are trying to train more.

"I would like to say that these young black kids are going to go into the community and make a difference," Littleton says. "But we need everybody." ■

Paul Smaglik is a freelance writer in Milwaukee, Wisconsin.

Raul Dela Cadena supports work on health disparities.

SLOW PROGRESS ON RESEARCH FUNDING

US organizations wishing to hire minority biomedical researchers face a battle after years of flat government research funding.

Tiffany Gary, an epidemiologist at Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, had ample publicly funded opportunities as an undergraduate. But many of the programmes for which she served as a mentor haven't contacted her for follow-up information or new assignments.

Foundations such as the Howard Hughes Medical Institute (HHMI) still offer minority mentorships. And although Elizabeth Ofili, associate dean of clinical research at the Morehouse School of Medicine in Atlanta, Georgia, notes that funding is tight, institutions specializing in minority education find ways to do more research. Morehouse has gained two or three researchers a year via National Institutes of Health (NIH) career-development awards. And the university works with non-minority doctors, as long as 30% of their patients are from minority groups.

Temple University in Philadelphia is using the NIH's R25 programme, which gives short-term training and emphasizes minorities in health research. Last summer, 14 Temple students took part. "We need to educate the principal investigators to use their research to focus on areas that may be related to health disparity," says Raul Dela Cadena, an assistant dean at Temple's medical school.

R25 is a feeder into another NIH programme for postdocs, the T32 grants. Universities like them because they bring in research funding. Leonor Corsino, a fellow at Duke University Medical Center in Durham, North Carolina, used a T32 to secure her current post and her school received a supplement for hiring someone from a minority group. Duke's minority recruitment and retention committee are what attracted Corsino: "People here at Duke were really interested in getting a minority in the department." But other Latino researchers are few and there are no African American fellows. That doesn't look likely to change, unless funding for minority training and recruitment increase. **P.S.**

Elizabeth Ofili: infrastructure needed.

NATURE|Vol 452|20 March 2008

MOVERS

Mike Tyers, director of the Scottish Universities Life Sciences Alliance, Edinburgh, Scotland

1995–2007: Senior investigator, Samuel Lunenfeld Research Institute, Toronto, Canada
1992–2007: Professor, Department of Medical Genetics and Microbiology University of Toronto, Canada

Mike Tyers is a world-renowned expert in cell division and a true believer in the notion that science can change the world. Both perspectives will inform his new duties as director of an alliance of Scotland's six major research universities.

The Scottish Universities Life Sciences Alliance is the latest of the pooling initiatives funded by the Scottish government. (Six other initiatives exist in areas ranging from engineering to geosciences.) The universities of Aberdeen, Dundee, Edinburgh, Glasgow, St Andrews and Strathclyde will share resources for life-sciences research. The Scottish Funding Council has put up £27 million (US$53 million) and the six universities another £50.6 million to create new research posts and to invest in core technology platforms. By pooling resources in strategic areas such as cell, systems and translational biology, Scotland can encourage collaboration across institutions and maximize productivity, says Tyers. "A relatively modest investment can go a long way."

The opportunity to direct a countrywide strategy for research is the main attraction for Tyers. One big priority will be promoting synthetic biology, which he sees as a logical evolution of systems-level approaches such as functional genomics and chemical biology.

After earning his doctorate in biochemistry from McMaster University in Canada, Tyers did postdoctoral work at Cold Spring Harbor Laboratory in New York. He took a faculty position at the University of Toronto in the Department of Medical Research and then settled in at the Samuel Lunenfeld Research Institute, also in Toronto.

Tyers developed his early fascination with the cell into a career-long research focus. He calls it his "cell-cycle-centric view of the world". Cell division affects "virtually every process you can think of", he says.

"He has a really clear vision of what ought to be done," says Bruce Futcher, who was Tyers' postdoctoral adviser and is now at Stony Brook University, New York. Tyers sets a high standard and leads by example, adds former student Paul Jorgensen, now a postdoctoral fellow at Harvard University.

Tyers likes to cite Shakespeare's King Henry V as a model for leadership. "It's imperative to get in the trenches, and let everyone know you're going to work as hard as anyone in the group," he says. "You can do an awful lot with a relatively small team of committed people." ■

Jill U. Adams

NETWORKS & SUPPORT

Sea change in business studies

A Master of Business Administration (MBA) degree may not be an obvious complement to oceanography research skills, but the combination could help to unearth novel economic solutions to ecological problems such as fisheries collapse. To those ends, the Scripps Institution of Oceanography (SIO) and the Rady School of Management at the University of California, San Diego, have created a joint oceanography PhD and MBA — the first in the United States.

"Ecology is the economics of nature," says SIO ecologist George Sugihara. "It is the flow and allocation of resources." Both are complex interconnected systems; perturbing one part causes ripples in another. "Management and policy are bumping up against business concerns, and having credentials in both worlds will give an individual that much more gravity," he says.

Damien Cie is the first to enrol in the programme, which starts this autumn. He wants to combine marine science, political science and anthropology to find environmentally friendly aquaculture approaches in his native Hawaii. "If I'm going to go out and tell scientists that the science is sound, I should also be able to demonstrate to business people why this is economically viable," he says.

Sugihara also expects areas such as marine-products chemistry, marine geology and biotechnology to benefit. The programme evolved out of an initiative from the Gordon and Betty Moore Foundation in San Francisco to create a pipeline of environmental conservationists with science and business backgrounds — which their non-governmental partners were finding difficult to recruit. Market research showed nothing similar.

JoAnne Starr, assistant dean of Rady, says it will serve as a model for any future interdisciplinary degree offerings there. "Business schools are typically isolated," she says. "We felt the need to establish one that would work in partnership with science and technology experts on campus to better reflect how innovation reaches the marketplace." Students must first be admitted to the SIO through the normal admissions process. They may apply to Rady the year before they hope to begin their MBA study.

Sugihara says the programme will attract a fairly special individual, given the rigorous qualification standards at both the SIO and Rady. But he has no doubt that the programme will yield innovative successes, combining marine excellence with business in ways that no one can yet anticipate. ■

Virginia Gewin

POSTDOC JOURNAL

Thinner air

I am a *ferengi* (foreigner) and my lungs are on fire. I must look as if I'm under the influence of some drug — I'm coughing and wheezing and have no appetite. And yet I have a permanent, beatific smile of wonder on my face. We have finally arrived at our campsite in the Simien Mountains of Ethiopia, more than 3,000 metres above sea level. I have been walking with the local baboons, called geladas, and I'm as high as a kite.

Just a few short days ago, my outlook was far more fatalistic and the future looked rather hopeless. Without the help of long-time Ethiopian friends in Addis Ababa, I am not sure how we would have escaped the city at all. To obtain a car, legal status within the country and amendments to agreements ad infinitum, we had to get signatures, stamps and approvals from a group of friendly but rather particular individuals spread across this large city. Small mistakes and misunderstandings would take days to correct. Escaping the city became an obsession. But nothing happens fast in Africa. Everybody but you has time.

Two long weeks later, we managed to head into the highlands. Now, with the shortage of oxygen to my brain, and panoramic views that make the Grand Canyon look like a dusty little upstart, I am experiencing a kind of euphoria. Bring on the monkeys, bring on the research questions, bring on the quest for scientific excellence! Nothing can bring me down — except perhaps gravity. ■

Aliza le Roux is a postdoctoral fellow in animal behaviour at the University of Michigan.

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IECB

Project leader positions at IECB Bordeaux

The European Institute of Chemistry and Biology, " Institut Européen de Chimie et Biologie " (IECB) is a recently created research institute sponsored by the University of Bordeaux, CNRS and INSERM. It is located on the University campus of Bordeaux in the Southwest of France. The Institute already hosts 16 research groups working at the interface of chemistry and biology in a new building (6000 sq meters) with state of the art equipment and facilities. The scientific policy of the Institute is under the responsibility of an International Scientific Board which is responsible for the selection of new group leaders.

IECB is recruiting PROJECT LEADERS

- ***IN BIOORGANIC or BIOMEDICAL CHEMISTRY***
  We seek a chemist to develop a research program aiming at exploring biological processes using the tools of organic synthesis. or in the area of protein polymer engineering or other polymer biomaterials.
- ***IN BIOMATERIAL CHEMISTRY***
  We seek an organic or polymer chemist interested in the area of synthetic polymer biomaterials or protein polymer engineering.
- ***IN MOLECULAR CELL BIOLOGY***
  We are interested in projects on macromolecules or processes in either the field of gene expression or in signal transduction.
- ***IN STRUCTURAL BIOLOGY (CRYSTALLOGRAPHY, NMR)***
  We seek biochemists working on structure/function relationship of biological macromolecules or on molecules of biomedical interest in particular in the field of cancer or in neurobiology.

We are looking for motivated young scientists who will demonstrate a strong potential for the development of an ambitious research programme. The applicants are expected to run independent and creative projects. They should be opened to interdisciplinary collaborations with other groups of the Institute.

Following the evaluation by the International Scientific Board of IECB, the candidate might be asked to apply for start-up funding from CNRS (ATIP) or INSERM (Avenir) programmes. Successful candidates will benefit of strong financial support from the Institute (post-doctoral salary and/or PhD fellowship) for the first two years. They will have access to state of the art facilities in structural biology, chemistry, molecular and cellular biology.

The applicants should be fluent in English. Knowledge of French would be useful but not mandatory. Applicants are invited to submit a detailed biography together with a research project and a list of potential referees to : Dr Jean-Jacques Toulmé, Scientific Director of IECB – 2 rue Robert Escarpit 33607 Pessac Cedex France e-mail: toulme@bordeaux.inserm.fr http://www.iecb.u-bordeaux.fr Deadline for applications: May 18, 2008.

W128109R

addex Pharmaceuticals

Addex Pharmaceuticals discovers and develops allosteric modulators, small molecule therapeutic agents that may offer more sophisticated ways to normalize biological signaling perturbed by disease compared to classical "orthosteric" agonist or antagonist drugs.

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- **CNS Diseases Therapeutic Area:** Laboratory Heads and Technicians in in vitro Pharmacology, Behavioral Pharmacology and Medicinal Chemistry.
- **Metabolic Diseases Therapeutic Area:** Group Leader, Laboratory Head, Technician
- **Clinical Development:** Clinical Operations Manager, Clinical Trial Manager
- **Non Clinical Development:** Head of Chemistry, Manufacturing and Controls

For more details, check our website: http://www.addexpharma.com

W128207R

Inserm

Postdoctoral position on pathophysiology of muscle disorders
INSERM U582/Institut de Myologie, Paris, France

Expertise on human genetics and/or molecular biology, including positional cloning, SNPs genome screen analysis and gene screening, is required.
The 18-month position is available immediately.

Applications should be sent to Dr. Ana FERREIRO
(a.ferreiro@institut-myologie.org)

W128261R

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UNIVERSITY OF BASEL, DEPARTMENT OF PHYSICS

Three Faculty Positions in Experimental Physics

(a) Nano Technology / Quantum Optics
(b) Nano Physics / Atomic, Molecular and Optical Physics
(c) Condensed Matter Physics

The Department of Physics at the University of Basel invites applications for two tenure-track assistant professorship positions and one associate or full faculty position. We seek interactive and creative scientists conducting outstanding experimental research that complements, strengthens and expands our activities in condensed matter physics and in particular nanophysics (http://physik.unibas.ch). Possible research directions include (but are not limited to): quantum optics, atomic, molecular and optical physics, soft and hard condensed matter physics, nano materials or scanning probe physics. For all positions, strong synergies with at least some of the Departments existing groups and activities in nano physics would be advantageous.

The successful applicants are expected to establish strong, independent research programs participating actively in the Swiss Nanoscience Institute (SNI) hosted at our Department as well as the Basel QC2 Center for Quantum Computation and Quantum Coherence. The ideal candidates will play an active role in our physics degree program as well as our recently established nano science curriculum teaching at all levels.

Applicants should provide a curriculum vitae, a publication list indicating five outstanding papers, a statement of research interests, a statement of teaching interests and experience, together with the names and addresses of five potential referees to: **Prof. Dr. Hans-Peter Hauri, Dean, Faculty of Science, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland,** and electronically (pdf or zip) to Marianne.Hess@unibas.ch.

The deadline for receipt of the application is May 31, 2008, but applications will continue to be considered until the positions are filled. The University of Basel is an equal opportunity employer and would like to particularly encourage applications from highly qualified female candidates. For additional information please contact any of the faculty members in condensed matter physics, http://physik.unibas.ch

UNI BASEL

W127698R

Ateliers de formation Inserm
Information and registration
101 rue de Tolbiac
75654 Paris Cedex 13 France
Tel: 33 (0) 144.23.62.04
Fax: 33 (0) 144.23.62.93
ateliers@tolbiac.inserm.fr

Inserm

French Institute
of Health and Medical Research

Atelier de formation 187
Analysis of DNA replication and genome instability using DNA combing and other single-molecule assays

Organizers: Jean-Sébastien Hoffmann (IPBS, Toulouse), Philippe Pasero (IGH, Montpellier), Etienne Schwob (IGMM, Montpellier)

● **Phase I**
Critical assessment…: June 26-27, 2008 ● Saint-Raphael

Aims
Single-molecule techniques have proven to be powerful approaches to monitor DNA replication in a variety of organisms, including human cells. Unlike other replication assays, these techniques allow the direct visualization of individual replication forks progressing along single chromosomes. Recent evidence indicates that spontaneous replication defects represent a major source of genomic instability in precancerous lesions and play a central role in the cancer process. The recent development of single-molecule approaches opens new avenues to understand the nature of replication stress in tumor cells and for the design of original anticancer strategies targeting the replication forks. The most advanced version of these techniques, called DNA combing, has been developed in France, and is still used by a limited number of laboratories. There is now a strong demand in France and abroad for a wider use of this technique. The aim of the workshop is to provide to the participants the latest methodological aspects of DNA combing and similar approaches and to discuss their respective benefits and shortcomings in light of the recent conceptual progress on genome replication and stability. The goal is to master these techniques for fundamental research on DNA replication and for applications in cancer research.

Audience
Researchers, technicians, postdocs and students interested in the « 3R » pathways (Replication, Repair, Recombination) in different model systems and their role in genetic instability of cancer cells. The workshop is also open to researchers from pharmaceutical companies.

Lectures will be given in English.

Programme
1. Single-molecule assays (DNA combing; SMARD; stretching of DNA and chromatin fibers; electron microscopy analysis of replication forks; capillary fibers and biophysics of single DNA molecules)
2. Analysis of DNA replication in model systems (Yeast; Xenopus; Mammalian Cells)
3. Applications of single molecule assays to fundamental and applied cancer research

● **Phase II**
Practical course: 6-10 October 2008 ● Montpellier/Toulouse
Programme
The practical course will focus on the DNA combing technology and its applications for studying DNA replication in yeast and human cells: in vivo labeling of replication origins; preparation of genomic DNA plugs, silanization of coverslips; combing and crosslinking of DNA fibers; immunodetection and image acquisition; statistical analysis of the data.

Selection
12 participants per theme will be selected among participants to phase I.

With the participation of : Aaron Bensimon (Paris, France), Michelle Debatisse (Paris, France), Olivier Hyrien (Paris, France), Dean Jackson (Manchester, UK), Massimo Lopes (Zurich, Switzerland), Raymond Monnat Jr (Seattle, USA), Paolo Norio (New York, USA), Marie-Jeanne Pillaire (Toulouse, France), Christophe Place (Lyon, France), Yves Pommier (Bethesda, USA), Nick Rhind (Worcester, USA), Jean-Louis Viovy (Paris, France).

Registration deadline: April 11, 2008

W123508A

Ateliers de formation Inserm
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ateliers@tolbiac.inserm.fr

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French Institute
of Health and Medical Research

Atelier de formation 186
Functional in vivo imaging methods: from molecules to cells

Organizers: Angela Giangrande, Michel Labouesse (IGBMC, Strasbourg)

● **Phase I**
Critical assessment…: June 5-6, 2008 ● Saint-Raphaël

Aims
Microscopy, which is an essential tool in developmental biology and neurobiology, has witnessed some major changes over the past ten years. Technological changes, with the development of ever more powerful microscopes, cameras and acquisition systems. Changes also in approaches, with the development of numerous fluorescent proteins, and their use in a wide array of methods relying on fluorescence, such FRAP, FRET, FLIM, FCS, FSM, etc… Physicists and cell biologists using tissue culture cells have undoubtedly been at the forefront in bringing up these changes. Their implementation, however, remains a challenge in live organisms, due to specific growth conditions to maintain them alive, due to problems arising from accessibility, phototoxicity, auto-fluorescence, or endogenous movements. The prime objective of the workshop will be to illustrate the most powerful strategies and approaches used to visualize biological processes in their dynamic aspects within live animals or tissues, regarding gene expression, transport of molecules/particles/vesicles, cell migration, junction rearrangements, cell division, calcium waves. Another objective will be to stress which techniques are available in live microscopy, what are their limitations, the predictable evolutions, and to review methods that allow to best overcome technical constraints attached to each experimental system.

Audience
Developmental biologists (in a broad sense), cell biologists using animal model systems, neurobiologists analyzing subcellular events, and more generally anyone who would like to get a good overview of what is possible using imaging approaches in animal models.

Lectures will be given in English.

Programme
Calcium imaging, gene expression in utero, cell migration, ribonucleoprotein particles transport, visualization of vesicles, movement of surface receptors within the plasma membrane, cytoskeleton remodeling. Ex vivo and explant culture, FRAP, FRET, fast imaging, spinning disk confocal microscopy, biosensors, quantum dots, multiphoton microscopy.

● **Phase II**
Technical workshop: Sept. 15-19 ● Paris/Strasbourg

Programme
Strasbourg: time-lapse with Drosophila and C. elegans embryos and larvae, montage demonstration and pupe preparation.
Paris: time-lapse with mice embryos ex-utero.

Selection
12 participants will be selected among participants to phase I.

With the participation of: Claude Antony (Heidelberg, Germany), Jérôme Collignon (Paris, France), Ilan Davis (Edinburgh, UK), Leanne Godinho (Munich, Germany), Stephan Thiberge (Princeton, USA), Thomas Lecuit (Marseille, France), Erez Raz (Göttingen, Germany), William Schafer (Cambridge, UK), Marcos Gonzalez-Gaitan (Geneva, Switzerland), Stephan Sigrist (Göttingen, Germany), Antoine Triller (Paris, France), Jean-Luc Vonesch (Strasbourg, France), Cornelius Weijer (Dundee, UK), Joachim Wittbrodt (Heidelberg, Germany).

Registration deadline: April 4, 2008

W123507A

The Center for Genomic Regulation (CRG, http://www.crg.es/) is a leading genomics research institute, associated with the University Pompeu Fabra (UPF) and located at the Parc de Recerca Biomèdica de Barcelona (PRBB, http://www.prbb.org/). The CRG contains six research programmes: Gene Regulation, Differentiation and Cancer, Cell & Development Biology, Systems Biology, Genes and Disease and Bioinformatics & Genomics, and has a partnership with the EMBL through the Systems Biology programme. The PRBB includes three other institutions devoted to biomedical research: the Department of Life and Health Sciences of the UPF (CEXS/UPF, http://www.upf.edu/cexs/), the Municipal Institute of Medical Research (IMIM, http://www.imim.es/) and the Centre for Regenerative Medicine of Barcelona (CMRB, http://www.cmrbarcelona.org/). To give support to this scientific community the CRG has built state of the art Genomics and Light microscopy facilities, as well as Screening and FACS facilities. New developments contemplate a top of the art proteomics facility.

GENOMICS CORE FACILITY

The Genomics Core Facility is committed to providing services in genotyping, next generation sequencing and microarrays and bioinformatics. Several positions are available:

Head of the Bioinformatics Core Facility – Ref. HBCF-0308

The Bioinformatics Core Facility will provide services both to the Genomics Core Facility and its users. The head of this unit will manage a team of several bioinformaticians. Responsibilities will include: defining the computational needs of the core facilities in collaboration with the heads of these units and the head of the scientific computational network, providing adequate bioinformatics support to both the core facility groups and their users, providing training for the bioinformatics solutions being deployed at the CRG. The head of bioinformatics may also be involved in research projects making use of the core facilities. The ideal candidate will be a PhD in biology or computer science, will have a solid background in computational biology and will have at least five years experience as a bioinformatics users support.

We offer an open-ended contract subject to periodic evaluations by an external advisory committee. The salary will be competitive, depending on experience, and equivalent to that of group leaders. Candidates should send a CV with list of publications, a brief proposal for the structure of the facility and the addresses of at least 3 potential references to: **rrhh@crg.es**

Biostatistician/ DataMiner – Ref. BDM-0308

One position is available to provide analysis support for microarray, genotyping and next generation sequencing data. Applicants should have a PhD (biostatistics, statistics, mathematics), with several years of experience in the field. Methodological research opportunities are available.

Bioinformatician – Ref. BIOINF-0308-1

One bioinformatician is needed to manage the data generated by the Genome Analyzer 1G (Solexa, Illumina) and GS-FLX (454, Roche) next generation sequencing instruments. The ideal candidate will be a computer scientist, or bioinformatician, with at least 2 years of relevant experience.

Bioinformatician – Ref. BIOINF-0308-2

One position is needed to provide expert assistance in microarray data analysis and to develop customized applications for automated processing, genome mapping and functional annotation of microarray data. A degree in Computer Science, Physics or Bioinformatics or related fields, with at least 2 years of relevant experience.

All these positions are funded by the CRG and the salary will be determined according to the education and experience of the candidates. Candidates should send a full CV and the addresses of at least 2 potential references to: **monica.bayes@crg.es**

CRG SCIENTIFIC COMPUTATIONAL NETWORK

The CRG is currently setting up a scientific computational network. For this purpose, we are seeking to employ the following positions:

UNIX system administrador	**- Ref. USA-0308**
Scientific Web Master	**- Ref. SWB-0308**
Database Administrador	**- Ref. DBA-0308**

Their collective task will be to set-up and maintain a scientific computational network designed for two Research Groups (Systems Biology and Bioinformatics & Genomics) and our rapidly expanding Core Facilities (Genotyping, Next Generation Sequencing, Microarrays and Proteomics facilities). The team responsible for the network will also be in charge of the development of web services associated with research and service activities. English is the working language within the institute. Candidates should send a full CV and the addresses of at least 2 potential references to: **romina.garrido@crg.es**

Detailed profile descriptions for the positions are available on www.crg.es
Applications Deadline: 8 weeks after the publication of this add.

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CEF Macromolecular Complexes Cluster of Excellence Frankfurt

JOHANN WOLFGANG GOETHE UNIVERSITÄT FRANKFURT AM MAIN

Professorship in Electron Optics/ Electron Cryo-Microscopy

The Cluster of Excellence "Macromolecular Complexes" and the Physic Department at the Goethe University in Frankfurt is seeking to appoint an outstanding scientist in the field Electron Optics/Electron Cryo-Microscopy. Three-dimensional cryo-EM is a key technique in our Cluster. We are looking for a new senior or junior professor to reinforce and complement ongoing research in the Cluster into the structure and molecular mechanisms of membrane proteins, transport machineries, RNA-protein complexes, or in related areas. Candidates should have an excellent track record in the structure determination of macromolecular assemblies by single-particle cryo-EM, electron tomography, and/or in instrument development. The new professorship will be structurally integrated into the Department of Physics.

Start-up funds as well as substantial funding for personnel und running costs are available. There will be ample opportunities for interactions with leading research groups at the University and the Max Planck Institutes of Biophysics and Brain Research. Excellent, state-of-the-art core facilities, including imaging, proteomics, genomics, and bioinformatics will be available to all groups in the Cluster. For further information, including the positions filled in the first round of appointments, see: www.cef-mc.de. Participation in new or existing DFG-funded Collaborative Research Centres (SFBs) in Frankfurt is expected. The successful candidate will develop a highly competitive research program in an area related to the interests of the Cluster and participate in teaching in Biophysics at the Bachelor's and Master's level.

The designated salary for the position is based on "W" on the German university scale or equivalent. The Goethe University is committed to a pluralistic campus community through affirmative action and equal opportunity. For details see: www.uni-frankfurt.de/aktuelles/ausschreibung/professuren/index.html.

Applications including a curriculum vitae, pdf files of 5 key publications, statements of research achievements (1 page) and future plans (3 pages), a record of teaching activities and third party-funding as well as the names and addresses of 5 academic referees should be sent **via e-mail by 10th April 2008** to: **Director, Cluster of Excellence Macromolecular Complexes Goethe University, Frankfurt, e-mail office@cef-mc.de**

www.uni-frankfurt.de

The University of Nottingham

Postdoctoral Research Fellow

CBS Centre for Biomolecular Sciences

Salary will be £25,134 per annum. This post will be offered on a fixed-term contract for a period of three years.

Further information is available at: http://jobs.nottingham.ac.uk/SCI365X1.

Candidates should send a detailed CV, together with the names and addresses of two referees, to Ms J Doughty, Centre for Biomolecular Sciences, School of Pharmacy, The University of Nottingham, University Park, Nottingham NG7 2RD. Email: Janeh.Doughty@Nottingham.ac.uk.

Please quote ref. SCI/365X1. Closing date: 9 April 2008.

This is a re-advertisement and previous candidates need not apply.

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http://jobs.nottingham.ac.uk

FCT Fundação para a Ciência e a Tecnologia

FUNDAÇÃO CALOUSTE GULBENKIAN

Champalimaud Foundation

GULBENKIAN/CHAMPALIMAUD PHD PROGRAMME IN NEUROSCIENCE: BIOLOGICAL BASES OF BEHAVIOUR

Call for Applications

Applications from independent thinkers with curiosity, creativity and drive from all backgrounds are sought to join the Gulbenkian/Champalimaud PhD Programme in Neuroscience at the Instituto Gulbenkian de Ciência (IGC), outside Lisbon, Portugal. Successful applicants will demonstrate ability to tackle great intellectual challenges, to learn new skills and ways of thinking, and to work passionately as part of a research team. Predoctoral training in quantitative disciplines (e.g. physics, mathematics, computer science), biological sciences (e.g. biology, medicine, bioengineering) or related fields is important. Previous research experience is desirable but not required.

Ten selected students will receive one year of intensive training led by distinguished researchers from around the world, participating in hands-on projects, symposia and workshops. Topics of instruction will include molecular biology and biophysics, ecology and evolution, development and learning, sensory and motor systems, as well as computational and cognitive neuroscience, ensuring a broad foundation for innovative and transdisciplinary work in basic or applied neuroscience.

The IGC is a dynamic international research environment hosting 200 scientists and PhD students in diverse fields of biology and biomedicine. This PhD Programme is part of a new and growing research programme – the Champalimaud Neuroscience Programme at the IGC, with a focus on understanding the neural circuits and systems underlying cognition and behavior. Portuguese nationals will also be eligible to perform research at select laboratories throughout the world.

Full tuition and stipend for 4 years of study for successful applicants is available through support by the Fundação para a Ciência e a Tecnologia (FCT), the Champalimaud Foundation, and the Gulbenkian Foundation. **The deadline for applications is 4 April 2008 for the class entering autumn 2008.** Complete application procedures and programme information are available at **http://pgcn.igc.gulbenkian.pt**

W127929R

Applications are invited for a young dynamic organic chemist as a Postdoctoral Fellow

To join the Animal Imaging Center- PET research group of the Center for Radiopharmaceutical Science of ETH, PSI and USZ located at ETH-Hönggerberg campus in Zurich. Research in this group focuses on the synthesis, radiolabelling with short-lived radionuclides such as carbon-11, fluorine-18 and the pharmacological characterization of selective and high affinity bioorganic molecules for the PET (positron emission tomography) imaging of tumors and brain function.

The successful candidate will participate in on-going and new research projects and will perform the synthesis and radiolabelling of potential pharmaceuticals for diagnostic imaging with PET.

The position will be for two years in the first instance and may be renewable.

For additional information please contact Prof. Dr. Simon M. Ametamey Tel. +41446337463 or secretary no. +41446337492
Applications including a CV should be sent to:
jolanda.steiner@pharma.ethz.ch
or
Ms Steiner Jolanda
Center for Radiopharmaceutical Science of ETH, PSI and USZ
ETH-Hönggerberg, D-CHAB IPW HCI H433
Wolfgang-Pauli-Str. 10
CH-8093 Zurich
Switzerland

W127905R

www.naturejobs.com

Tenure Track/Tenure-Eligible Investigator

The Neuro-Oncology Branch (NOB) of the National Cancer Institute, component of the National Institutes of Health, is calling for applications for a Tenure-Track/Tenure-Eligible Investigator. The overall goal of the NOB is to develop novel therapeutic strategies for the treatment of primary brain tumors through an understanding, exploitation, and eventual clinical translation of the principles underlying the molecular and genetic pathogenesis of these tumors. Our approach is to leverage the unique resources of the intramural NIH program, including its tremendous scientific and clinical freedom to explore high risk yet high pay off projects, to build an NIH-wide pre-clinical and clinical brain tumor experimental therapeutics center. NOB works collaboratively and synergistically with both the NIH extramural community as well as with the private sector to ensure the most efficient and rapid development of novel approaches to the treatment of these devastating tumors. In order to accomplish these goals, the NOB is trying to identify candidates with a broad knowledge of basic cancer biology, clinical oncology and a strong motivation for translational research in brain tumors with a particular emphasis on gliomas.

The successful candidate must have an MD or MD/Ph.D. Degree and should have substantial experience (documented by an extensive publication record) in one or more of the following fields: signal transduction mechanisms, gene discovery, therapeutic target development, molecular/cellular cancer biology, cancer genetics and genomics. A preference will be given to physician scientists with a strong background in clinical medical oncology/neuro-oncology and with an emphasis in translational research. The candidate is expected to develop his/her own line of research to complement and enhance the current efforts of the NOB towards elucidating the biology of gliomas and alleviating the suffering of patients with brain tumors through the development of innovative therapeutic approaches.

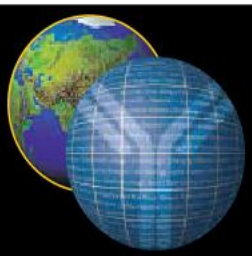
Salary will be commensurate with experience.

Interested applicants should submit:

- A letter of indicating their interest in the position
- A statement of research interests
- A career synopsis
- Current curriculum vitae and complete bibliography
- Names and addresses of five references

Applications should be postmarked by **July 11, 2008** to: **Dr. Kevin Camphausen, Chair, c/o Karen Abraham, Neuro-Oncology Branch Search Committee, Bloch Building, MSC 8200, Room 237, 9030 Old Georgetown Road, Bethesda, MD 20892.**

DHHS, NIH, the National Cancer Institute and the National Institute of Neurological Disorders and Stroke are Equal Employment Opportunity and Affirmative Action employers that value and foster diversity throughout the entire organization.



NIAID Needs You Because the World Needs Us

Staff Scientist - Nonhuman Primate Immunogenicity Core

The VRC brings together a diverse group of scientists with the goal of advancing vaccine-related research as well as initiating and advancing vaccine candidates through clinical trials. Crucial to the basic and preclinical efforts are immunological evaluations in the nonhuman primate (NHP) model. In terms of basic research, the VRC is actively involved in studying innate and adaptive immune responses, lymphocyte trafficking, the generation and maintenance of memory responses in systemic and mucosal sites, as well as pathogenesis following SIV infection. In translational research, the VRC is optimizing immunogenicity of a variety of different platforms and vectors by studying the effects of adjuvants, schedules, and delivery platforms for vaccine vectors. Finally, the VRC conducts preclinical testing of vaccines in the NHP model; these data are used to support moving clinical products forward to human testing and can be critical to regulatory filings.

To support these efforts, the VRC has created the NHP Immunogenicity Core (NIC). Currently, we are recruiting a Staff Scientist (Core) to lead the NIC. This individual will oversee all NIC operations, including (1) consulting on the design and implementation of NHP studies; (2) coordinating all studies, sample collection, and analysis with the VRC Laboratory of Animal Medicine, including writing and defending Animal Study Protocols before the Institutional ACUC; (3) developing standard operating procedures for, and validating, cellular and humoral immunogenicity assays; (4) implementing all testing according to GLP guidelines; (5) collating, analyzing, and coordinating all data; and (6) preparing oral and written presentations of studies for both internal and external use. In addition, the Staff Scientist will coordinate with VRC Principal Investigators to design, implement, and analyze immunogenicity and immunopathogenesis experiments for basic research projects.

We are looking for a highly experienced, motivated, and creative individual with a Ph.D. or M.D. and at least five years of postdoctoral experience to head the NIC. Candidates with experience in the NHP model and/or experience with GLP will have a significant advantage. The position is available immediately. Interested candidates should contact Mario Roederer for more information:

Mario Roederer
VRC, NIAID, NIH
40 Convent Dr., Room 5509
Bethesda, MD 20892-3015
301-594-8491; FAX 301-480-2788
Email: Roederer@nih.gov

To learn more about NIAID and to view additional job opportunities, please visit: http://healthresearch.niaid.nih.gov/vrc

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National Institutes of Health
Department of Health and Human Services

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OPPORTUNITIES @ NIH THE NATIONAL INSTITUTES OF HEALTH

THE NIH IS DEDICATED TO BUILDING A DIVERSE COMMUNITY IN ITS TRAINING AND EMPLOYMENT PROGRAMS

WPI Immunology Frontier Research Center, Osaka University, Japan

Professors/Associate Professors and Postdoctoral Researchers Positions

WPI Immunology Frontier Research Center (WPI-IFReC), Osaka University has been launched on October, 2007. The main theme of this research center, headed by Dr. Shizuo Akira, director of WPI-IFReC, is "Studies on immunological dynamics by in vivo imaging technologies". The aim of the research is to unveil the whole picture of a dynamic immune system by employing a variety of imaging technologies.

(1) Professors/Associate Professors

We are seeking Professors/Associate Professors who establish research groups in following two main topics:
1. Immunological research based on in vivo imaging technology
2. Systems Biology/Bioinformatics in immunology

Each research group is provided with start-up budget, support personnel, excellent laboratory environment and equipment. Your salary will be highly competitive, commensurate with level of your experience and background. Initial term of position is for five years with possible extension.
Applicants should submit following documents by email to General Affairs Section, WPI-IFReC.
E-mail:ifrec-office@ifrec.osaka-u.ac.jp
CV, publication list, summary of research (approx. 1000 words), detailed outline of future research proposals (approx. 1000 words), list of research grants acquired in the last five years, three letters of recommendation

(2) Postdoctoral Researchers

Applicants should choose a research group where you would like to join from laboratories listed on our website:
http://www.ifrec.osaka-u.ac.jp/eng/laboratory/index.php
Your salary will depend on your qualification and experience, with a minimum of $44,000 per year. Initial term of position is for three years.
Applicants should submit following documents by email to General Affairs Section, WPI-IFReC.
E-mail:ifrec-office@ifrec.osaka-u.ac.jp
CV, publication list, names of three references

Positions available after April 2009. Applications for (1) and (2) must be completed by **30 June, 2008**.

Contact:
WPI Immunology Frontier Research Center, Osaka University
3-1 Yamada-oka, Suita, Osaka 565-0871, Japan
E-mail: ifrec-office@ifrec.osaka-u.ac.jp
For more information about WPI-IFReC, please visit our website at:
http://www.ifrec.osaka-u.ac.jp/index-e.php

JP127808R

Director Positions

Institute of Cellular and Organismic Biology and Institute of Plant and Microbial Biology Academia Sinica, Taiwan

Academia Sinica, Taiwan, invites applications and nominations for the positions of Director of Institute of Cellular & Organismic Biology (ICOB) and Director of Institute of Plant and Microbial Biology (IPMB). The initial appointment is for a period of three years (renewable for a second term), and will also carry the title of Research Fellow.

Academia Sinica is the pre-eminent academic institution in Taiwan. It is devoted to basic and applied research in mathematics and physical sciences, life sciences, and humanities and social sciences. ICOB currently engages in the following research areas: Integrative studies in mechanisms of animal adaptation, animal viruses and microbiology, and animal models and drug screening. IPMB's research ranges from plant molecular and cell biology, genetics, physiology, pathology and systematics. Two Academia Sinica Research Stations, Marine Research Station and South Taiwan Agricultural Research Station, are available for Academia Sinica researchers. Both institutes are well funded and equipped with modern research facilities. For details about Academia Sinica and ICOB and IPMB, please consult the website: http://www.sinica.edu.tw

Interested candidates should have a Ph.D. degree, a distinguished record of academic scholarship, and diverse experience in university and professional service. He/she is expected to pursue a vigorous research program. The successful candidates will be expected to build on the existing strengths of the respective institutions, develop new research thrusts, promote basic life sciences and provide intellectual leadership in relevant basic and applied life sciences in Taiwan.

Applications and nominations, including complete curriculum vitae, a publication list, and three letters of recommendation, should be submitted to **Dr. Andrew H.-J. Wang, Vice President, Academia Sinica, 128 Academia Road Section 2, Nankang, Taipei, 115, Taiwan** or by email to: searchvp@gate.sinica.edu.tw. Screening of applications/nominations will begin immediately, and will continue until the positions are filled.

JP127676R

Junta de Castilla y León

Centro de Láseres Pulsados Ultracortos Ultraintensos (CLPU)

SENIOR MANAGER FOR DIRECTOR OF THE SCIENTIFIC RESEARCH FACILITY

'Ultra-short, Ultra-intense Pulsed Laser Centre (CLPU)'

The Ultra-short, Ultra-intense Pulsed Laser Centre (clpu) is a new research facility that has been created as a Consortium of the Spanish Ministry of Education and Science, the Regional Government of Castile & Leon and the University of Salamanca, as part of the implementation of the Spanish Scientific Infrastructures Roadmap. The Consortium headquarters are located in Salamanca, Spain. The Consortium was created on 19 December 2007.

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The objectives of the Consortium are:

- To build and operate a Petawatt Laser in Salamanca.
- To develop ultra-short-pulse technology in Spain
- To make significant advances in intense, compact laser technology
- To promote the use of such technology in several fields: Physics, Engineering, Chemistry, Biology, Medicine, Energy, etc.
- To open the facility to the domestic and international scientific community.

The Ultra-short, Ultra-intense-pulse Laser Centre (CLPU) seeks a DIRECTOR

The Director will report to the Governing Council and is responsible for managing the construction and operations, and for maximizing its readiness and effectiveness for scientific research. The Director will be responsible for recruiting and maintaining high-quality scientific, technical and administrative staff, developing an annual budget for review and approval, and proposing the short-and long-range plans for the Centre.

The Director will be responsible for scheduling and implementing the construction and operation of the Ultra-short, Ultra-intense Pulsed Laser Centre, and will act as the primary interface with potential scientific and industrial users. The Director will maintain an effective liaison with the Council and its Executive Committee, together with the Advisory Committees. The Director will also be responsible for the general maintenance of the Centre and its laboratories, for maintaining a public outreach office and for pursuing and managing public and private fund-raising activities, with guidance from the Council.

Candidates should have a profile in senior management of the construction and operations of research infrastructures in the field of interest for CLPU, preferably with a scientific background in this area, experience in the administration and management of publicly funded scientific facilities of research centres, as well as in staff management.

The salary range and starting date are negotiable. A review of applications will begin in may 2008, and recruitment will remain open until the position is filled.

Applications together with the names of two referees should be submitted by e-mail to the Chair of the Search Committee (dgpt@mec.es, sgpitgi@mec.es, munabagr@jcyl.es)

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wellcome trust sanger institute

Wellcome Trust Sanger Institute

Pathogen Group

Bacterial Genomics vacancies

Two Postdoctoral Fellow positions in Bacterial phylogenetics and Genomics of the human microbiota are available within the Pathogen group at the Wellcome Trust Sanger Institute. A Senior Computer Programmer is also required. Please refer to our website www.sanger.ac.uk for more details

Contact

Human Resources
Email: recruit@sanger.ac.uk
http://www.sanger.ac.uk

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JLINK SERVICES
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Crescent University, Nigeria

Academic Positions

Crescent University, an Islamic and Science based university in Southern Nigeria requires professors, senior lecturers and lecturers in its Biological, Mathematical, Chemical and Physical Sciences departments. For professors and senior lecturers PhD is essential. Lecturers need MSc or PhD. All positions require teaching experience.

Contact

Helen Green
Email: h.green@jlinkservices.co.uk
http://www.jlinkservices.co.uk

A new Helmholtz Centre in Berlin

HELMHOLTZ GEMEINSCHAFT

MATERIALS AND ENERGY

Two of Berlin's largest Research Centres - the Hahn-Meitner-Institut and BESSY - are going to merge by January 2009 to a new Helmholtz Centre and will be a member of the Helmholtz Association - Germany's largest scientific organisation.

We invite applications for four postdoctoral positions in the Berlin Neutron Scattering Centre BENSC at the Hahn-Meitner-Institut Berlin, Germany. The Hahn-Meitner-Institut is a German National Laboratory and a member of the Hermann von Helmholtz Association of National Research Centres and has about 800 employees. It hosts the Berlin Neutron Scattering Center (http://www.hmi.de/bensc/), a user facility open to scientists from all over the world. To probe the structure and dynamics of solids and liquids, BENSC provides the national and international research community with state-of-the-art neutron scattering equipment and expertise.

Department of Magnetism

4 scientists PH.D.

(physicist, chemist, crystallographer)

Reference no. SF 2008/3

The successful candidate will take part in the research program of the department which includes major activities in quantum and frustrated magnetism and will undertake responsibilities at our thermal triple-axis spectrometer E1 (http://www.hmi.de/bensc/). A broad knowledge in condensed matter physics or chemistry is highly desired as well as experience in scattering methods. A background in neutron or x-ray scattering and strong scientific interest in magnetism will be preferred.
For informal inquiries, please contact Prof. Alan Tennant (phone: +49-30-8062-2741, e-mail: tennant@hmi.de) or Dr. Norbert Stüsser (phone: +49-30-8062-3171, e-mail: stuesser@hmi.de).

Reference no. SF 2008/5

The successful candidate will take part in the research program of the department which includes major activities in quantum and frustrated magnetism and will undertake user service responsibilities at the flat-cone diffractometer E2 (http://www.hmi.de/bensc/). A broad knowledge in condensed matter physics or chemistry is highly desired as well as experience in scattering methods. A background in neutron or x-ray diffraction, crystallography and strong scientific interest in magnetism will be preferred.
For informal inquiries, please contact Prof. Alan Tennant (phone: +49-30-8062-2741, e-mail: tennant@hmi.de), Dr. J.U. Hoffmann (phone: +49-30-8062-2185, e-mail: hoffmann-j@hmi.de) or Dr. Norbert Stüsser (phone: +49-30-8062-3171, e-mail: stuesser@hmi.de).

Reference no. SF 2008/7

The successful candidate will join the Novel Materials Group and take part in the research program (http://www.hmi.de/people/argyriou), whose activities focus on strongly correlated magnetic oxides such as multiferroic manganites, sodium cobaltate as well as frustrated magnetic systems. She/he will take responsibility for the High Resolution Powder Diffractometer E9 (http://www.hmi.de/bensc/). The candidate will be expected to organize the user experimental program, coordinate technical support and provide support for users on E9 (30% of time). A broad knowledge in condensed matter physics or chemistry and a background in neutron or x-ray scattering, magnetism, crystallography or solid-state synthesis methods are highly desired. Staff at BENSC have access to 30% of the beamtime on all neutron scattering instruments and to internal beam time at the HMI beam lines at the BESSY synchrotron for their own research.
For informal inquiries, please contact Dr. Dimitri Argyriou (phone: +49-30-8062-3016, e-mail: argyriou@hmi.de).

Reference no. SF 2008/6

The successful candidate will take part in the research program of the department which includes major activities in novel materials, superconductivity and magnetism and will undertake user service responsibilities at the small angle neutron scattering instrument V4 (http://www.hmi.de/bensc/). A broad knowledge in condensed matter physics or chemistry is highly desired as well as experience in diffraction. A background in neutron or x-ray scattering techniques and strong scientific interest in superconductivity or magnetism will be preferred.
For informal inquiries, please contact Dr. Uwe Keiderling (phone: +49-30-8062-2339, e-mail: keiderling@hmi.de) or Dr. Norbert Stüsser (phone: +49-30-8062-3171, e-mail: stuesser@hmi.de).

All four appointments are for 3 years. Applicants should send a letter of application, a detailed CV, two academic references, a list of publications and copies of degrees to Hahn-Meitner-Institut Berlin, Abt. Personal und Soziales, Glienicker Str. 100, D-14109 Berlin, Germany, quoting the respective reference no. for the position (e-mail: personalabteilung@hmi.de). The deadline for all applications is 15th April 2008.

W128208R

hmi www.hmi.de

UPPSALA UNIVERSITET

Chair in Theoretical Chemistry

at the Department of Physical and Analytical Chemistry, Programme of Quantum Chemistry at the Ångström Laboratory

Within theoretical chemistry, the department presently offers postgraduate courses and research opportunities in applied quantum chemistry, theoretical chemical physics and computational chemistry. The subject is broad and comprises areas such as solar cells, radical chemistry, exotic molecules and anti-atoms, quantum information theory and numerical methods for quantum dynamics. Electronic structure calculations are central. Uppsala University is looking for a person who can further strengthen the research area towards chemistry and molecular science or else establish a new area. Collaboration with experimental activities within chemistry is also a qualification.

The tasks will include: Comprehensive responsibility for research and postgraduate studies in theoretical chemistry, teaching and advising PhD-students and undergraduate students. Research in theoretical chemistry. Information about research and development and planning. of new research projects. Administration at a divisional or higher level.

For further information about the position, please contact professor Kristina Edström, tel +46 (0)70 167 9006

Information about the Section of Chemistry can be found at www.chemistry.uu.se and about the Department of Physical and Analytical Chemistry, Quantum Chemistry at www.kvac.uu.se.

Further particulars including instructions for applicants can be obtained from Anital.Ljungstrom@uadm.uu.se. This information can also be found at http://www.teknat.uu.se/english/index.php. Closing date for acceptance of applications is April 21, 2008.

www.uu.se

W128203R

HelmholtzZentrum münchen

German Research Center for Environmental Health

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

The Medical Faculty of the Ludwig Maximilians University München and the Helmholtz Zentrum München - German Research Center for Environmental Health (HMGU), invite applications for a position as a

Professor (W3) of Genetic Epidemiology and Director of the HMGU-Institute of Genetic Epidemiology

The position is permanent, the employment will be at Ludwig Maximilians University.

Tasks are teaching Genetic Epidemiology at the University and leading the HMGU - Institute of Genetic Epidemiology. The focus of the position is on planning, realization and analysis of genetic epidemiological studies of complex disease. Collaboration with LMUinnovativ and the Munich Center of Health Sciences is expected. Also participation in the postgraduate curriculum Public Health and Epidemiology is intended.

Requirements for employment are a university degree, teaching ability, doctorate, and habilitation or equivalent scientific expertise. Candidates with training in statistics and experience in the application of epidemiological/statistical methods in genetic research are preferred.

The successful candidate cannot be older than 52 years at the time of appointment. Exceptions to the age limit can be made in urgent cases. Priority will be given to physically disabled persons with equivalent qualifications.

Ludwig Maximilians University and Helmholtz Zentrum München both wish to increase the proportion of women in research and strongly encourage qualified female candidates to apply.

Applications with curriculum vitae, references, certificates, list of publications, reprints of the most significant publications, and a short research proposal should be sent to the Dean of the Medical Faculty of LMU Prof. Dr. D. Reinhardt, Bavariaring 19, D-80336 München, Germany.

Please also provide us with an electronic version of your application via e-mail: wichmann@helmholtz-muenchen.de. Deadline for the application procedure is April 30, 2008.

www.helmholtz-muenchen.de **www.uni-muenchen.de**

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RUBICON

Applications for membership

RUBICON Network of Excellence (http://rubicon-net.org/)

RUBICON is funded by the European Commission as a European forum for research on the molecular principles and regulatory roles of protein modification by linkage of ubiquitin or ubiquitin-like molecules. RUBICON brings together investigators from different backgrounds to work in a multi-disciplinary effort to develop the knowledge base that may facilitate the discovery of novel mechanism-based therapeutic approaches to disease.

Junior investigators

that will complement RUBICON's expertise in the field are invited to apply. Candidates with research program focusing on quantitative biology, systems biology, and rational drug design are specifically encouraged to apply.

The new members will have access to all RUBICON events, research funding and joint resources, and will be expected to collaborate with RUBICON partner laboratories.

Selection criteria: Scientific excellence will be the sole criterion for selection. A "junior investigator" is defined as being maximally 5 years after establishment as an independent research group.

Informal enquiries can be made to the Rubicon coordinator, Prof. Maria Masucci, email: maria.masucci@ki.se

To apply, please send application, including a covering letter, CV, list of publications, and a concise research proposal (max 5 pages) by email to Javier.Avila-Carino@ki.se, **before June 15, 2008.**

Rubicon, Prof. Maria G. Masucci, CMB, Karolinska Institutet, Box 285, SE-171 77 Stockholm, Sweden

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FCT Fundação para a Ciência e a Tecnologia

FUNDAÇÃO CALOUSTE GULBENKIAN

IGC INTERNATIONAL PhD PROGRAMME IN MULTIDISCIPLINARY LIFE SCIENCES (PGD)

Call for applications

Applications are now open for the 2008 academic year PhD Programme in Multidisciplinary Life Sciences, organised by the Instituto Gulbenkian de Ciência (IGC) with support from the Fundação para a Ciência e a Tecnologia (FCT).

From September 2008, 10 students, supported by FCT fellowships, will receive 3 months' graduate training and education, by an international faculty, followed by 3 and a half years research at an IGC group or in collaboration with external groups, in Portugal or abroad.

The PGD is a programme for highly motivated and independent thinkers with a strong interest in Life Sciences, who aspire for excellence and team work while engaging in innovative and multidisciplinary research. We seek candidates who are not afraid to work hard and are able to overcome scientific challenges. A range of backgrounds in the life sciences are welcome (biology, biochemistry, biological engineering, pharmaceutical sciences), as well as other fields such as physics, computer science and mathematics.

The IGC is a world-leading centre for multidisciplinary research in Life Sciences. The institute hosts 30 international research groups engaged in different aspects of life science research, offering a multidisciplinary environment where both computational and experimental approaches are pursued. Research programmes span several areas, namely evolution, epidemiology, cell and developmental biology, immunology, genetics, molecular basis of complex human diseases, systems biology, neurobiology.

Portuguese and foreign candidates will have successfully completed a minimum of 4 years higher education (for Europeans, 240 ECTS) by September 1st, 2008. Applicants will be selected for interview conducted either in person or remotely, by phone or videoconferencing. Accepted students will be expected to start the programme in September 2008.

The deadline for applications is 4 April 2008.

Detailed information on the programme and complete application procedures are available at **http://www.igc.gulbenkian.pt/node/view/32.**

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DPZ

Deutsches Primatenzentrum

The Leibniz Gemeinschaft is a consortium of actually 82 science and service institutes for research activities facilitated by the Federal and Länder authorities.

The German Primate Center GmbH (DPZ) as a member of the Leibniz Community conducts scientific and biomedical research on and with primates.

Within the Department of Primate Genetics the staff position as a

Bioinformatician

is available for initially five years. The applicant is expected to establish an own work group focussing on the areas sequence and genome analysis and / or phylogeny in primates.

A master´s degree in bioinformatics, biology (with profound knowledge of bioinformatics) or graduation in informatics with post doc experience is required. We expect profound knowledge in bioinformatic analysis of large DNA sequence data sets and of mammalian genomes, the construction of data banks and in the application of phylogenetic programmes supplemented by a short exposé (max. 3 pages) about future research projects.

Applications of women are specifically desired. The employment at the DPZ is carried out according to the regulations of the public services. The payment matches TV-L. Handicapped applicants with equivalent qualifications will be given priority.

Written applications with the customary documents are to be submitted until April 15th, 2008 to the German Primate Center GmbH, Leibniz Institute for Primate Research, Personalstelle, Kellnerweg 4, 37077 Göttingen or via email (bewerbung@dpz.eu).

Further information about the DPZ and the research group "primate genetics" can be obtained at http://www.dpz.eu or via telephone: +49 551 3851 161.

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Further information about the Leibniz Gemeinschaft please visit the following link:
http://www.leibniz-gemeinschaft.de

Leibniz Gemeinschaft

Career articles from Naturejobs

- **Naturejobs Prospect**: quick takes on career implications of current events
- **Naturejobs Special Report**: examinations of jobs issues on both sides of the bench
- **Naturejobs Careers & Recruitment**: discipline-by-discipline exploration of opportunities
- **Naturejobs Regions**: tours of scientific hubs
- **Naturejobs Movers**: traffic reports that follow high-profile scientific globe-trotters and sector-hoppers
- **Naturejobs Postdocs & Students**: guides to taking a step toward a permanent position

www.naturejobs.com

naturejobs making science work

nature publishing group npg

World leader in the development of breakthrough treatments.
Committed to innovation.
New medicines through strong science.
Think what's possible.

A global healthcare leader, Novartis has one of the most exciting product pipelines in the industry today. A pipeline of innovative medicines brought to life by diverse, talented, performance-driven people. All of which makes us one of the most rewarding employers in our field.

The Novartis Gastrointestinal Disease Area aims to discover novel therapies for the treatment of functional GI disorders and inflammatory bowel diseases. After establishing full drug discovery capabilities over the last two years, we are looking for a talented and motivated scientist to extend our mucosal biology group.

Laboratory Head Horsham, Sussex

We are looking for a high performing motivated scientist with PhD and postdoctoral experience to join our mucosal biology group as a Laboratory Head. With a background in epithelial biology or inflammation and a strong interest in signal transduction, ideally with gastrointestinal experience, you will lead a small team and contribute to research programmes discovering new drugs to treat inflammatory bowel diseases and other GI disorders.

To find out more about this and other opportunities – and the benefits of working for Novartis – please visit www.nibrcareers.com quoting the reference 37093BR.

NOVARTIS
INSTITUTES FOR BIOMEDICAL RESEARCH

www.nibrcareers.com

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PRIFYSGOL ABERYSTWYTH UNIVERSITY

POSTDOCTORAL RESEARCH ASSOCIATE IN MACHINE LEARNING & DRUG DESIGN

DEPARTMENT OF COMPUTER SCIENCE
£21,682 - £32,796

Applications are invited for the post of postdoctoral Research Associate on the BBSRC funded project "A Robot Scientist for drug design and chemical genetics" at Aberystwyth. This work is in collaboration with Prof. Steve Oliver at Cambridge. The successful applicant will have a relevant background experience in machine learning and/or drug design. The work will involve the new Robot Scientist "Eve". This post is available for a fixed-term of three years and is available immediately.

Informal enquiries may be made to Prof. Ross D. King rdk@aber.ac.uk

Ref: CS.08.06. Closing date: 15 April 2008.

For further particulars and an application form please go to www.aber.ac.uk. Tel: 01970 628701. Email: vacancies@aber.ac.uk

Bilingual Institution which operates a Welsh Language Scheme.
Committed to Equal Opportunities.

U128117R

University of Oxford

Mathematical, Physical and Life Sciences Division
Department of Chemistry

University Lecturership and Associated College Tutorial Fellowship

Physical and Theoretical Chemistry Section in association with Magdalen College.

The Department of Chemistry proposes to appoint a University Lecturer in the Physical and Theoretical Chemistry Section with effect from 1 October 2008 or as soon as possible thereafter. The Lecturership will be associated with a Tutorial Fellowship at Magdalen College. The Selection Committee hopes to make the appointment in experimental Physical Chemistry and welcomes applications in all areas of physical chemistry and its allied fields.

The combined University and College salary for each post will be on a scale currently up to £52,628 p.a. (increases are due in May and October 2008). Additional College allowances are available as set out in the further particulars.

Further particulars, containing details of the application procedure and of the duties, may be obtained from a) the Jobs Vacancies section of the Departmental website http://www.chem.ox.ac.uk/jobs.asp or b) by writing to Professor Gus Hancock, Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ. E-mail: laura-jean.holdcroft@chem.ox.ac.uk
Closing date is 24 April 2008.

The University of Oxford and Magdalen College are Equal Opportunities Employers

U128231R

www.ox.ac.uk/jobs

Barts and The London
School of Medicine and Dentistry

William Harvey Research Institute
Non-Clinical Lecturer/Senior Lecturer

(Ref: 08073/JO)/ (Ref 08074/JO)

£30,968-£34,518 or £38,515-£45,469 per annum (including London Weighting)

The newly formed Centre for Microvascular Research at the William Harvey Research Institute aims to develop an internationally recognised group to investigate the molecular interactions that mediate and regulate inflammatory and vascular events within the microcirculation. The Centre, headed by Professor Sussan Nourshargh, will build upon the group's established reputation as a leader in the field of leukocyte trafficking and will use a multidisciplinary approach for addressing its objectives. The Centre is based on the Charterhouse Square Campus of Barts and The London School of Medicine and offers newly refurbished laboratories and cutting edge imaging facilities in the heart of the City of London.

Candidates should be established independent scientists with proven track record of research productivity in terms of high calibre publications/fund raising and capable of making a significant contribution to the overall objectives of the group. Experience in research into inflammatory events, vascular biology and/or immunology and expertise in molecular/cell biology, imaging and/or in vivo methodologies would be advantageous.

The benefits package for these posts includes:

- 30 days leave plus 4 College closure days
- Childcare vouchers scheme
- Contributory final salary pension scheme
- Interest free season ticket loan

For an application form and further information, please visit the Human Resources website on http://www.hr.qmul.ac.uk/vacancies, or request details (quoting the above reference numbers) via email csq-recruit@qmul.ac.uk. Applicants wishing to make a non-electronic application may contact our 24 hour recruitment line on 020-7882 6149 for details.

Informal enquiries about these posts can be made to Professor Nourshargh, email: s.nourshargh@qmul.ac.uk.

The deadline for return of completed applications is **12 noon (BST) on Friday 4th April 2008.** Applications (quoting the above reference numbers) should be returned via email to csq-recruit@qmul.ac.uk. Alternative means of applying are available; please contact the recruitment line on 020-7882 6149 for details. Completed applications must not be sent directly to the Centre for Microvascular Research or to Professor Nourshargh.

Interviews are likely to be held in the week commencing 21st April 2008.

Promoting excellence in teaching, learning and research
Working towards Equal Opportunities

U127876R

dkfz.

German Cancer Research Center
(DKFZ, Deutsches Krebsforschungszentrum) Member of the Helmholtz-Gemeinschaft, HGF

Faculty of Biosciences
University of Heidelberg

The mission of the German Cancer Research Center (DKFZ) is to unravel mechanisms of cancer development and to establish novel approaches for the diagnosis, treatment, and prevention of cancer. As a leading biomedical research center in Germany, our programs focus on basic and translational cancer research. The German Cancer Research Center is a foundation under public law and a member of the Helmholtz Association of National Research Centers (Helmholtz-Gemeinschaft Deutscher Forschungszentren).

The following position is available at the German Cancer Research Center (DKFZ) in cooperation with the Faculty of Biosciences at the University of Heidelberg

Professorship (W3) and Division Head for Cellular Immunology

We are seeking a candidate with an outstanding research record and international reputation, who studies fundamental questions in immunology, continuing a tradition of excellence in this discipline at DKFZ. The research program of the successful candidate is expected to strengthen the existing research program "Tumor Immunology" of the DKFZ. Candidates should hold a PhD or MD degree. The successful candidate will have the possibility to participate in graduate and postgraduate programs of the Faculty of Biosciences at the University of Heidelberg.

The professorship is tenured. First professorial appointments are limited in time before tenure can be granted. DKFZ is committed to increase the percentage of female scientists and encourages in particular female applicants. Among candidates of equal aptitude and qualifications, a person with disabilities will be given preference.

Applications, including curriculum vitae, list of publications, and a research program, should be sent to Prof. Dr. Otmar D. Wiestler, Chairman, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany (e-mail: o.wiestler@dkfz.de) not later than May 2, 2008.

W128121R

"Advertising an open high profile position Nature jobs resulted in outstanding group of highly qualified applicants from all over the world within a very short period of time. This efficient service is our first choice for scientific recruitment"

Dr Obrecht Jean-Pierre,
Polyphor AG, Switzerland

Search **over 5,000*** scientific vacancies on
naturejobs.com

*Publisher's data 10 March 2008

nature publishing group npg

Inflammation at Interfaces

Inflammation research represents one of the fastest growing academic disciplines with a large potential for translation into novel diagnostics and therapies. The universities of Kiel and Lübeck, the Leibniz Center for Medicine and Biosciences (Borstel) and the Max Planck Institute for Evolutionary Biology (Plön), have established the multi-disciplinary research network (since November 2007: Cluster of Excellence) "Inflammation at Interfaces". Focus is the study of mechanisms of chronic inflammatory diseases of barrier organs (e.g. lung, skin, intestine).

The DFG Cluster of Excellence provides superb infrastructures and career perspectives to young researchers. In different thematic areas are immediately available

9 Positions for Young Group Leaders

within three Integrative Research Networks in the Cluster of Excellence.

The Integrative Research Network **"Cytokine Signaling via gp130"** seeks
4 Postgraduate Scientists to lead Independent Junior Groups

Candidates are expected to have an excellent research record in the respective field and to be willing to contribute to one of these five major research areas:

- Structural and molecular design of novel cytokine antagonists.
- Invertebrate model organisms.
- Novel transgenic animal models for chronic inflammatory and infectious diseases.
- Infection driven inflammation models (i.e. experimental models of Tuberculosis and Chagas Disease).
- Pharmacogenomics of gp130 in human Crohn disease.

Inquiries will be welcomed by Prof. S. Rose-John, e-mail rosejohn@biochem.uni-kiel.de

The Integrative Research Network **"NOD-like Receptors"** seeks
4 Postgraduate Scientists to lead Independent Junior Groups

Candidates are expected to have an excellent research record in the respective field and to be willing to contribute to one of the major research areas of the Integrative Research Network:

- Phylogeny and evolution of genetic variability in NLRs.
- Structural biology of NLRs and their cognate ligands.
- Phagolysosomal compartments and PAMP recognition.
- Sequence variants in NLR genes and human inflammatory diseases.

Inquiries will be welcomed by Prof. P. Rosenstiel, e-mail p.rosenstiel@mucosa.de

The Integrative Research Network **"Autoimmunity to Type VII Collagen"** seeks
1 Postgraduate Scientist to lead an Independent Junior Group

The candidate is expected to have an excellent research record in the respective field and to be willing to contribute to the major research areas of the Integrative Research Network (hosted in Borstel):

- Inflammatory pathways in autoimmunity to type VII collagen.
- Autoimmune driven animal models.
- Innate immune effector functions and their related signalling pathways.

Inquiries will be welcomed by PD Dr. F. Petersen, e-mail fpeters@fz-borstel.de or Prof. D. Zillikens, e-mail detlef.zillikens@uk-sh.de

The Cluster of Excellence offers state-of-the-art research facilities and a supportive multi-disciplinary environment including established models for molecular biological research, animal facilities and infection models. The positions will be hosted in different academic institutions throughout the cluster (Kiel, Lübeck, Borstel).
Candidates are expected to have a PhD as well as an excellent research record in the respective field. Salary depends on legal and personal qualification and experience of the successful candidate and will be up to grade E14 (TV-L). Contracted hours are currently at 38.7 hours/week.
Younger postdocs are offered their own laboratory in association with one of the existing research groups. Positions (approx 3 years initial contract) can be extended for up to 5 years. Experienced postdocs will be offered to lead an independent junior research group including a budget for personnel, equipment and consumables.
Information about the Cluster of Excellence 'Inflammation at Interfaces' can be found at http://www.inflammation-at-interfaces.de.
Applications are accepted preferably as PDF including a covering letter, CV, list of publications, a summary of scientific achievements and a short three-year research plan as well as three references with contact information.
The institutions are equal opportunities employers.
The state government and the institutions support the employment of disabled persons. Persons with disabilities will, with appropriate qualifications and aptitudes, be employed preferentially.
The institutions offer a family-friendly working environment and are proactive with respect to double-career families. The institutions strongly encourage women with appropriate qualifications to apply for the positions. Women with equivalent qualifications, competence and expertise will be given preference.

Closing date is 3 weeks after publication of the advertisement. Applications should be marked with the thematic area (e.g. "gp130") and sent to:

Dr. Ina Plettner
Cluster of Excellence "Inflammation at Interfaces"
Kiel University
Christian-Albrechts-Platz 4
D-24098 Kiel
Germany
Fax : ++49 (0)431 8801560
e-mail: iplettner@uv.uni-kiel.de

CAU Christian-Albrechts-Universität zu Kiel

UKSH

W128031R

nature REVIEWS CANCER
www.nature.com/reviews/cancer

Associate Editor

Nature Reviews Cancer has a vacancy for an Associate Editor.

This exciting position involves working closely with the Chief Editor and other members of the journal team on all aspects of the editorial process, including commissioning and editing reviews, organizing peer-review, writing for the journal, and developing the content of the journal, both in print and online.

To meet these challenging tasks, the ideal candidate will have a broad knowledge of cancer biology and/or therapy, and hold a PhD in a relevant field. We are particularly interested in applicants with at least 2 years of postdoctoral experience, but applications from outstanding candidates who have recently completed their PhD are also welcome. A key aspect of the job is liaising with the scientific community and attending international conferences, so the successful candidate must be dynamic and outgoing with excellent interpersonal skills. Previous editorial experience would be an advantage, but is not essential.

The position will be based in the London office of Nature Publishing Group, and the terms and conditions are highly competitive, reflecting the importance and responsibilities of the role.

For further information about the Nature Reviews series visit http://www.nature.com/reviews

To apply, please send your CV, a summary of relevant experience, and current salary, quoting reference number **NPG/LON/842** to: Denise Pitter, Personnel Assistant, at londonrecruitment@macmillan.co.uk

Closing Date: Thursday 27th March 2008

nature publishing group npg

IN127887R

University of Oxford

Department of Cardiovascular Medicine
Wellcome Trust Centre for Human Genetics

Postdoctoral Scientist

£26,666 - £32,796 p.a.

We are looking for a motivated postdoctoral fellow to join our research group studying the role of myocardial high-energy phosphate metabolism in the development of cardiac hypertrophy and heart failure. The post involves the biochemical characterisation of the cardiac phenotype of transgenic models of perturbed creatine and phosphocreatine content.

You will be well-organised and able to work independently and contribute conceptually to the overall research programme of our multidisciplinary group.

You will have demonstrable expertise in the biochemistry of cellular energy metabolism and experience in working with heart preparations and in nuclear magnetic resonance (NMR) in biological samples would be an advantage. This British Heart Foundation-funded post is initially for two and a half years with the possibility of extension thereafter and is available immediately.

Informal inquires about the group's research activities can be made to the Department of Cardiovascular Medicine. Further particulars, which detail the application procedure, should be obtained from: http://www.admin.ox.ac.uk/fp/ or e-mail: enquiries@cardiov.ox.ac.uk Please quote reference V8/SN/011. The closing date for applications is 11 April 2008. Interviews will be held as soon as possible after the closing date.

As an Equal Opportunity employer, we positively encourage applications from people of all backgrounds

U128232RM

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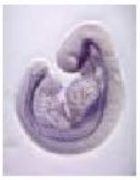
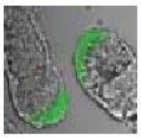
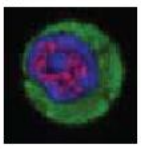
Research Group Leader Integrative Biology

MRC Clinical Sciences Centre







Applications are invited for a group leader position at the MRC Clincial Sciences Centre located on the Hammersmith Campus of Imperial College in West London. Candidates will have an outstanding publication record and the ambition to develop an internationally competitive research programme using integrative approaches to biological systems. Potential research areas include transcriptional networks, computational biology, genomics, proteomics bioinformatics and/or modelling with relevance to a fundamental aspect of biology or medicine.

The Clinical Sciences Centre is a direct funded, multidisciplinary MRC institute and fosters innovative and collaborative research. Particular strengths are in the areas of cell type specification, gene expression, development, and the genetic basis of disease.

A generous startup package is available for the successful applicant.

For more information visit our website: www.csc.mrc.ac.uk
reference number CSC08/140
Informal enquires can be made to
Professor Tim Aitman
email: t.aitman@csc.mrc.ac.uk
and Professor Matthias Merkenschlager
email: matthais.merkenschlager@csc.mrc.ac.uk

There is no formal deadline for applications although we are seeking to fill this position by end of 2008.

The MRC is an equal opportunities employer.

U128240R

University of Dundee

College of Life Sciences

College of Life Sciences and Wellcome Trust Biocentre, University of Dundee, Scotland

POSTDOCTORAL RESEARCHER
RESEARCH ASSISTANT/PROGRAMMER

The 5* research-rated College comprises 70 Research Groups and over 700 scientists and support staff from 53 countries and was rated one of the top five European Research Institutes in which to work ("The Scientist", November 2007). Research papers published by University of Dundee scientists in the fields of Biology and Biochemistry were more highly cited over the past 10 years than any other UK University.

We have a new postdoctoral vacancy with David Martin and a Research Assistant/Programmer is required to work with Geoff Barton. For further details about these positions and how to apply, please consult our website or email HR-LifeSciences@dundee.ac.uk

www.lifesci.dundee.ac.uk/

U128237R

"Through our posting for a post-doc position at NatureJobs we have received messages from a surprisingly large number of highly qualified investigators from all over the world, and have been able to recruit a suitable candidate. Thank you very much"

Alberto Sánchez-Fueyo, MD,
Hospital Clinic Barcelona/IDIBAPS, Spain

Innovation, multiculturalism and internationalisation. This is what Leiden University stands for. Since 1575, the university has made prominent contributions to the prosperity, well-being, and culture of society. All this in a climate where talent can be developed without ideological, cultural or religious restrictions. We offer a personal approach in a close-knit environment. Leiden University co-operates with other leading universities in the League of European Research Universities. Discover the freedom of academic spirit experienced every day by our students, faculty and staff. Discover Leiden University, an inspiring working environment with a strong academic tradition.

www.vacatures.leidenuniv.nl

Faculty of Science

Tenure Track / Faculty positions in Physics (2 x Full-time)

Vacancy number: 8-062

The Leiden Institute of Physics invites energetic scientists with outstanding qualifications and a research interest in one of the current topics of research at our Institute, to apply for a tenure track position in a challenging career path.

Tenure Track position in Theoretical Chemistry (Full-time)

Vacancy number: 8-068

The Leiden Institute of Chemistry invites energetic scientists with outstanding qualifications and a research interest in reaction dynamics and/or electronic structure to apply for a tenure track position in a challenging career path.

In a tenure track you will start your own independent research line and contribute to shaping the future directions of science research in Leiden.
The track consists of a temporary appointment of 6 years maximum, and is expected to lead to a tenured position as associate professor. A promotion to a position as full professor can be expected within the subsequent 3 to 5 years. Appointments at a higher entry level are possible for exceptionally qualified candidates.

More information on these openings can be found at our website www.vacatures.leidenuniv.nl.

Universiteit Leiden
The Netherlands

W128209R

Leiden University. The university to discover.

Medizinische Fakultät Carl Gustav Carus
Reformfakultät des Stifterverbandes
für die Deutsche Wissenschaft

TECHNISCHE UNIVERSITÄT DRESDEN

The **DFG-Research Center for Regenerative Therapies Dresden** focuses on fundamental research into regeneration, tissue engineering and stem cells. In a joint effort of several scientific institutions in Dresden, it forms a network of currently 80 research groups plus commercial partners, working in the areas of hematology/ oncology, diabetes, neurodegenerative disorders, bone/ cartilage replacement and cardiovascular disease. Available immediately is the following position:

Scientist for protein expression and antibody generation

We are seeking a highly motivated Post-doc, who will contribute to the analysis of the autoimmune response during diabetes. The Post-doc will clone IgG genes from single human B cells, express heavy and light chains in various expression systems and define the epitopes the antibodies recognise. In addition, the scientists will provide protein expression and antibody generation service to CRTD groups. He will express and purify proteins and generate antibodies in rabbits and/or monoclonal mouse antibodies. The applicant should have experience in molecular biology (cloning, PCR), protein expression in E. coli, yeast and tissue culture cells, protein purification and immunization of rabbits. Experience in the generation of monoclonal antibodies is also beneficial. The operating language of the institute is English.

For further information see **www.crt-dresden.de.**

Please send your application in English with self addressed, stamped envelope, under the **code number 025/2008** latest by **15.04.2008** to: **Technische Universität Dresden, Medizinische Fakultät Carl Gustav Carus, DFG-Forschungszentrum für Regenerative Therapien Dresden, Andrea Hempel, Fetscherstraße 74, 01307 Dresden, Germany, email: andrea.hempel@crt-dresden.de (only as one PDF document).**

W128212R

Max Planck Institute for Biology of Ageing

MAX-PLANCK-GESELLSCHAFT

The Max Planck Institute for Biology of Ageing in Cologne invites applications for the position of a

Scientific Administrator

The Scientific Administrator will take an active part in scientific and administrative activities necessary for the build-up phase of the new institute and the new building. You must be able independently to handle scientific administrative matters of the institute and should provide a link between the managing director and other parts of the science-related administration of the institute.

Your responsibilities as a Scientific Administrator will include monitoring funding opportunities and facilitation and coordination of grant applications, production of scientific reports and other Institute reports, organization of a Max Planck graduate program, teaching activities (lectures and practical courses), organization of meetings and symposia, liason with the University of Cologne and with Max Planck Central Administration, liaison with the City of Cologne and other stakeholders, coordination of publicity for the Institute, as well as coordination of the board of trustees and of the scientific advisory board. You will also supervise and carry out science-related administrative work for the departments of the Institute. You should be highly enthusiastic and committed and have a strong scientific background inculding a PhD or equivalent qualification.

The position requires high level scientific administrative skills and scientific knowledge and the ability to anticipate and be pro-active. You will have excellent communication and writing skills in German and English, and an enthusiasm for working as a member of an active team in a rapidly changing situation. You are expected to work closely with the directors of the institute, and to take responsibility for the interactions of the Institute with other organisations, particularly the University of Cologne.

The Max Planck Institute for Biology of Ageing offers an excellent research environment with other Max Planck Institutes and the University of Cologne (http://www.medizin.uni-koeln.de).

Your contract (up to TVöD 15 depending on experience and qualification) will be initially for a 5-year fixed-term.

The Max Planck Society is committed to employing more handicapped individuals and especially encourages them to apply.

Applications will be considered until the position is filled. Please send your application and CV in English and the names of 2-3 referees by 31. March 2008 via email to: balt@nf.mpg.de

W128204R

University of ULSTER

SCHOOL OF ENVIRONMENTAL SCIENCES

Research Fellow in Geophysics

Fixed-term for five years.

Salary: £35,836 – £36,912 Ref: C07/467/N
Closing date: 18 April 2008 Base: Coleraine

The University wishes to appoint a Research Fellow in Geophysics to undertake research in the Geophysics Research Group aimed at the development of geophysical capability in the area of characterisation and monitoring of potential sites for CO_2 sequestration. The post is funded by the Irish Department of Communications, Energy and Natural Resources under the Griffiths Geoscience Research Awards Scheme. The appointee will contribute to the expansion of research in Geophysics, and will collaborate with project partners in the Geophysics Group, University College Dublin. Applicants must hold a PhD in an appropriate area and have significant experience in conducting geophysical research.

GSI

NDP

Our preferred method of issuing application packs is via our website at

www.ulster.ac.uk/jobs

t: 028 7032 4072 e: jobs@ulster.ac.uk

The University is an equal opportunities employer and welcomes applicants from all sections of the community. Appointment will be made on merit.

U128242R

Online Editor, Protein Structure Initiative Knowledgebase

Nature Publishing Group is looking for a dynamic, organized and creative science graduate with a background in structural biology to launch and maintain the forthcoming Protein Structure Initiative (PSI) Knowledgebase. The successful applicant will also have a keen interest in and ideas for making the site accessible to a broad audience of molecular and cellular biologists as well as geneticists.

Launching in 2008, the Knowledgebase will be an accessible online publication widely read by the research community. The site will encompass editorial content updated monthly on recent research, news and events, as well as databases and other information resources from the PSI. The Knowledgebase is an innovative publication of a type that is becoming increasingly important in academic publishing, and we are looking for someone who is eager to establish the Knowledgebase as a major information resource for researchers.

The Editor will take responsibility for the site's content and high scientific quality, including writing summaries of key research developments. The editor will work as part of the existing teams in NPG's Web Publishing department and at *Nature Structural and Molecular Biology*, and will liaise with the PSI. They will have, or will be shortly expecting to receive, a PhD in a structural biology-related discipline, and will have a broad interest and understanding of the structural biology field, including technologies and their applications. A sound knowledge of good web practice and a passion for the exploitation of the medium as a means of scientific communication are crucial.

Key personal qualities for this position include:

- Excellent writing skills
- A strong ability to communicate with leading scientists
- An acute eye for detail, and the ability to work to firm deadlines

The successful candidate will ideally be based in our offices in New York, although other localities may be possible.

To Apply: Send cover letter stating salary requirements and resume via email to admin@natureny.com (Nature Publishing Group, Human Resources Department) no later than **April 14, 2008**. Note "Online Editor" in the subject header.

NPG is an Equal Opportunity Employer.

nature publishing group npg

IN128171R

100 Top Hospital expanding in Central Texas

TEXAS A&M INSTITUTE OF REGENERATIVE MEDICINE IN TEMPLE, TX.

Post-doctoral and Faculty Positions

The newly established Texas A & M Institute of Regenerative Medicine is seeking post-doctoral fellows and faculty for research on adult stem/progenitor cells. The Institute is dedicated to research both on the basic biology of stem/progenitor cells and their potentials for therapies of human diseases. It will occupy newly renovated laboratories and a series of core laboratories in an expanding academic medical center. Post-doctoral appointments will be for one year with the opportunity to renew for a second and third year subject to performance. Faculty appointments will be in an appropriate academic department and will range in rank from tenure-track assistant professors to tenured full professors depending on qualifications. Faculty appointments will include institutionally-funded salaries, start-up funds, modern laboratory space, and access to graduate students. Salaries and benefits are competitive. Candidates should have excellent verbal skills and a Ph.D. or M.D. degree from a well recognized university.

Before March 1, 2008, please send curriculum vitae, brief statement of research interests, indication of level of appointment sought, and three letters of recommendation to attention of Darwin J. Prockop, M.D., Ph.D., Director, Texas A&M Institute of Regenerative Medicine at email address: Regenerate@medicine.tamhsc.edu. The Texas A&M Health Science Center is an AA/EO Employer.

SCOTT & WHITE

Texas A&M Health Science Center College of Medicine

NW127477R

Assistant Editor

Nature Biotechnology

Nature Biotechnology seeks an Assistant Editor for its editorial team based in New York. Expertise in systems biology and/or computational biology would be desirable, but not required.

Members of the editorial team evaluate manuscripts, oversee the peer review process, commission and edit secondary materials such as Reviews, and write short pieces and editorials for the journal. The successful applicant will attend scientific meetings and visit laboratories to maintain contact with the international scientific community. The position will play a key role in consolidating *Nature Biotechnology's* presence in the fields of systems biology and computational biology.

Excellent communication skills and a willingness and ability to learn new fields are a must. Applicants should have completed a Ph.D. in the biological sciences.

To apply, an interested candidate should submit a curriculum vitae, a short (500-1000 words) News and Views-style article on an exciting and newsworthy recent development in biotechnology, and a cover letter explaining their interest in the position to Human Resources Department, Nature Publishing Group.

All applications should be sent via email to: admin@natureny.com.

Please place "Assistant Editor Nature Biotechnology" in the subject line.

All applicants will be reviewed upon receipt with a close date of March 31, 2008.

Nature Publishing Group
75 Varick Street
New York, New York 10013, USA

nature publishing group npg

IN125903R

Director of Research
Pediatric Neuromusculoskeletal Disorders

Shriners Hospitals for Children and the Temple University School of Medicine, Philadelphia, jointly invite applications and nominations for a Director to lead the Philadelphia Shriners Hospitals Center of Excellence in Pediatric Neuromusculoskeletal Disorders Research.

The director will guide development of a collaborative research program with an emphasis on spinal cord injury and neural repair. SHC will provide an attractive package for recruiting investigators at all levels and equip the laboratory over a five-year period. The 25,000-square-foot laboratory will be located within TUSM's new 11-story research and education building, providing access to the outstanding research resources of TUSM, as well as opportunities for extensive collaboration with the biomedical science community at TU and other major research centers. There will also be a strong alliance with the existing clinical research program at the Shriners Philadelphia Hospital. These things will be fundamental to the success of this program and will provide an ideal environment for a model translational research program.

The ideal candidate will be a senior scientist with a vigorous laboratory research program and sufficient experience and interests to provide intellectual and programmatic leadership. The position will be salaried by TU and will carry an academic appointment as a tenured Professor in the TUSM department that is best aligned with the Director's research interests.

For consideration, applicants should send a letter of interest and curriculum vitae by April 30, 2008 to: **Zakir Bengali, Ph.D., Corporate Director of Research Programs, Shriners Hospitals for Children, Rocky Point Drive, Tampa, FL 33607-1435.**

We are an equal opportunity/affirmative action employer and strongly encourage applications from women and minorities.

NW127973R

Massachusetts Institute of Technology

Faculty Positions

The MIT Nuclear Science and Engineering Department invites applications for faculty positions. Appointments would most likely be at the assistant or untenured associate professor level. In special cases, a senior faculty appointment may be possible.

The department is a world leader in the application of nuclear and radiation phenomena to engineering systems. Its faculty teach and conduct research in a broad range of areas from fundamental nuclear science to practical applications of nuclear technology in energy, health care, and other industries. The department's current activities encompass fission reactor technology; plasma physics and fusion technology; security technology; radiation physics; and biological, medical, and information sciences.

Applicants should have a doctorate in an engineering or physical sciences field and must have demonstrated excellence in research. A commitment to excel in teaching the foundations and applications of nuclear science and engineering is essential. The department is searching broadly and outstanding candidates working in any branch of nuclear science and engineering will be given serious consideration.

To apply, submit a curriculum vita, statement of research interests, and the names of three references via e-mail to nsefacultysearch@mit.edu or by mail to MIT, Nuclear Science and Engineering Dept., Faculty Search, Room E38-104, 77 Massachusetts Avenue, Cambridge, MA 02139-4307.

MIT is an equal opportunity/affirmative action employer. Women and minorities are encouraged to apply.

DEPARTMENT OF NUCLEAR SCIENCE & ENGINEERING

NW128048R

http://web.mit.edu

STANFORD UNIVERSITY

Climate Science Faculty Position

The School of Earth Sciences and the Woods Institute for the Environment solicit applications for a tenure-track faculty appointment in the area of climate science. We are looking for a person with a demonstrated research record who is also committed to quality undergraduate and graduate teaching. This position will be jointly held between the newly formed Department of Environmental Earth System Science and the Woods Institute for the Environment.

We seek a climate scientist who uses data and models to explore key elements and processes in the evolving global climate system. Areas of interest may include anthropogenic forcing of climate change, atmospheric processes, or interactions between climate and other parts of the Earth system. The successful applicant will have demonstrated interests in interacting effectively with a broad range of Stanford colleagues, including physical, chemical, and biological scientists as well as engineers and social scientists interested in policy implications for sustainability. The search is open to applicants at the assistant and associate professorial rank.

Stanford University is an equal opportunity employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as from others who would bring additional dimensions to the university's research, teaching and clinical missions.

Please apply online in electronic format (.pdf only) with the following application material: cover letter, curriculum vitae, a statement outlining research and teaching experience and interests, and the names and addresses of three or more referees, at http://pangea.stanford.edu/jobs/. Select the Climate Science faculty position.

Questions can be directed to Prof. Robert Dunbar (dunbar@stanford.edu) or Prof. Christopher Field (cfield@globalecology.stanford.edu).

Applications should be received by April 30, 2008, to assure full consideration.

NW127834R

BCM Baylor College of Medicine

Faculty Positions
Department of Pharmacology

The Department of Pharmacology is expanding and invites applications from outstanding scientists for several tenure track Assistant and Associate Professor positions. A competitive laboratory start up package will be provided to successful candidates to support the development of independent, funded research programs in pharmacogenomics, chemical biology, protein design and engineering, computational chemistry or other areas broadly relevant to pharmacology. Candidates should have a Ph.D. and/or M.D. degree and postdoctoral experience as well as a strong record of research accomplishments. Baylor College of Medicine is located in the Texas Medical Center and offers a highly interactive environment and a strong infrastructure for research. Review of applications will begin in March 2008 and continue until the positions are filled.

Applicants should submit a statement of research interests and curriculum vitae as a single PDF to pharmacology@bcm.edu. Three letters of reference should be sent separately to pharmacology@bcm.edu, attention: Timothy Palzkill, Ph.D., Department of Pharmacology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Baylor College of Medicine is an Equal Opportunity/Affirmative Action and Equal Access Employer

NW127862R

Division of Pulmonary, Critical Care, and Sleep Medicine Department of Medicine

Beth Israel Deaconess Medical Center
Boston, Massachusetts 02215

The Divisions of Pulmonary, Critical Care, and Sleep Medicine and Matrix Biology at Beth Israel Deaconess Medical Center are jointly seeking a basic researcher at the Assistant/ Associate Professor level with an interest in the lung matrix. Successful applicants will have an established record of independent research support, an aptitude for mentoring new investigators, and an interest in collaboration on translational projects with BIDMC's Interstitial Lung Disease Center. Academic rank will be determined by experience. Beth Israel Deaconess is a 590-bed regional and national referral center that is a major teaching hospital of Harvard Medical School.

Beth Israel Deaconess Medical Center and Harvard Medical School are Equal Opportunity Employers. Women and minorities are particularly encouraged to apply. Please send applications or nominations, together with a current curriculum vitae, to:

J. Woodrow Weiss, M.D.
Chief, Division of Pulmonary, Critical Care, and Sleep Medicine
Beth Israel Deaconess Medical Center
330 Brookline Avenue, GZ405
Boston, Massachusetts 02215

NW127997R

ASSISTANT DIRECTOR
Banbury Center

The Banbury Center at Cold Spring Harbor Laboratory, is world-renowned as a venue for small discussion meetings. The topics of the meetings range across all areas of current biological research–molecular biology & genetics; bioinformatics & genomics; neuroscience & science policy.

We are seeking a highly motivated, versatile individual to share responsibilities for the Center's program w/ Dr. Jan Witkowski, the Executive Director. You will have special responsibility for developing the program of meetings on disorders of mental health, w/ particular emphasis on genetic approaches.

Ph.D. or M.D. & at least three yrs exp of postdoctoral research in neuroscience, molecular biology or human molecular genetics req'd. Research exp in mental health related topics pref'd. Scientists working in editorial positions are also invited to apply. Grant prep exp preferred. Excellent writing skills are essential & applicants will be asked to submit samples of their work.

Further information about the Banbury Center & Cold Spring Harbor Laboratory will be found at www.cshl.edu/banbury & www.cshl.edu. ***Please send*** your CV & the names of three references to: E-mail: jobline@cshl.edu Fax: 516-367-6850. NW128151R

Cold Spring Harbor Laboratory
Human Resources • 1 Bungtown Road
Cold Spring Harbor, NY 11724 EOE

The University of Michigan

Chemistry Fellows program is seeking applications from outstanding individuals to become Michigan Fellows in Chemistry. This new postdoctoral program, housed in the Department of Chemistry, is geared towards exceptional scientists who are just completing, or have recently completed, their PhD studies. The program will combine a competitive salary ($50,000 annually for two years plus benefits), exciting research programs, and professional development opportunities specifically designed for each candidate.

The Department of Chemistry is home to a multi-faceted group of world-class researchers working on all aspects of modern chemistry. Research within the department is characterized by strong collaborations within Chemistry and with top-ranked engineering and medical schools. Ann Arbor is regularly ranked high on lists of desirable places to live. Cultural amenities in theatre, music and film rank with those of the nation's largest cities, while cost of living is substantially more affordable. In addition, the city is conveniently located within 20 minutes of the Detroit airport, and within a three-hour car ride from Chicago and Toronto.

Full details of the nomination and application process and the opportunities available in the Michigan Chemistry Fellows program can be found at: http://www.umich.edu/~michchem/fellows/. Questions about the program can be directed to: chemfellows@umich.edu. Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is a non-discriminatory, affirmative action employer.

NW127859R

FACULTY

NW128198R

The Penn Center for AIDS Research at the University of Pennsylvania School of Medicine seeks candidates for an Associate or Full Professor position in the tenure track. Rank will be commensurate with experience. The successful applicant will be accomplished in the area of international research in HIV/AIDS. Applicants must have an M.D. or Ph.D. or equivalent degree. We seek candidates who have an established international HIV/AIDS-related research program that may include, but is not limited to, areas of HIV transmission and pathogenesis; co-infections associated with AIDS; treatment or epidemiology. The Penn CFAR presently has an international AIDS research program in Botswana, and the applicant will be expected to play a leadership role in the research mission of the Penn CFAR at that site. The University of Pennsylvania has a broad base of AIDS investigators with vigorous programs in virology, pathogenesis, behavioral studies, immunology/vaccine development, an active ACTU, and a broad range of graduate programs. Brand-new laboratory space at the Botswana site is presently under development. Applicants must have established expertise in their area of research and a record of scientific independence. Faculty appointment will be in an appropriate department in the School of Medicine. The University of Pennsylvania is an equal opportunity, affirmative action employer. Women and minority candidates are strongly encouraged to apply. **Please email CV, cover letter, 3 reference names, statement of research interests as a pdf file to: olivere@mail.med.upenn.edu.** Dr. Susuan Ross, Professor of Microbiology, Chair of Search Committee. c/o Ms. Evelyn Olivieri, Assoc. Director, Center for AIDS Research, UPenn School of Med, 353 Biomedical Res'rch Bldg II/III, 421 Curie Blvd, Phila, PA 19104-6160. CFAR web: http://www.uphs.upenn.edu/aids/

POSTDOCTORAL POSITION

Available immediately to study the genetics of human aging using state of the art population genomics approaches in the Louisiana Healthy Aging Study (e.g. see Biometrics [2007] 63, 1245).
Send CV and 3 references to: S. Michal Jazwinski, PhD, Tulane Center for Aging, Tulane University Health Sciences Center, 1430 Tulane Ave.,SL-12, New Orleans, LA 70112. Electronic applications (sjazwins@tulane.edu) will receive prompt attention. AA/EOE.

NW128156R

Johns Hopkins University
Brain Sciences Institute
Postdoctoral Position

NW128144RL

The laboratory of Dr. Ahmet Hoke has a position available in drug development for peripheral neuropathies and nerve regeneration. Project req's exp in neuronal cultures and assay development. Medicinal chemistry exp pref'd. Candidate will interface with Johns Hopkins University High Throughput Screening Center and develop new approaches to drug screening.

Contact

Please send curriculum vitae and three references to email: ahoke@jhmi.edu

Univ. of Pittsburgh, PACCM
Cell/molecular biology
Post Doctoral/PhD

NW128035RL

Seeking applicants for NIH funded researcher with interest in cell/molecular biology of primary human epithelial cells. Join established multidisciplinary team in areas of advanced lung disease. Knowledge of nitric oxide pathways, asthma immunology preferred. Send letter and CV to: Sally Wenzel, MD , Div. of Pulmonary, Allergy, Critical Care Medicine. AA/EOE

Contact

UMPC Montefiore Hospital
NW 628 3459 5 Ave
Pittsburgh, PA 15213
Email: wenzelse@upmc.edu

naturejobs

Announcing the launch of *Naturejobs* in India

The opening of *Naturejobs* in India, as well as the coinciding launch of the *Nature India* website (**www.nature.com/natureindia),** enables recruiters in the region to target jobseekers more effectively.

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IN126638R

Max-Planck-Institut für Züchtungsforschung
Max Planck Institute for Plant Breeding Research

The Max Planck Institute for Plant Breeding Research (MPIZ) seeks a

Head of Bioinformatics and IT

to provide bioinformatics support to scientists and to lead the technical IT team. The successful applicant will provide high level bioinformatics support to research groups in the institute as well as training courses for graduate students. Furthermore, the appointee will coordinate system administration and bioinformatics and therefore have overall responsibility for computing support in the institute. Currently these groups comprise of a technical manager, one supporter and one administrator as well as 2 trainees and 2 scientific collaborators for the bioinformatics service. The appointee will also have the opportunity to pursue independent research for which extra resources may be available.

We seek a candidate with a PhD in biology, bioinformatics, computer science or a related field with very good English and preferably German language skills. Payment and benefits are according to the German TVöD. The position will initially be limited to five years with the possibility to make it permanent.

The Max Planck Institute for Plant Breeding Research (MPIZ) in Cologne (http://www.mpiz-koeln.mpg.de/) is one of the world's premier sites committed to basic research and training in plant science. The institute consists of four scientific departments, three independent research groups and specialist support, totalling about 400 staff, including externally funded positions.

The Max Planck Society is an equal opportunity employer.

Please send your detailed application by 18th April 2008 to:

Max Planck Institute
for Plant Breeding Research
Personnel Administration
"Head of Bioinformatics/IT"
Carl-von-Linné-Weg 10
50829 Cologne
Germany

MAX-PLANCK-GESELLSCHAFT

W128229R

Federal Ministry of Education and Research

bccn

"Bernstein Award" 2008

Young Scientists Research Award in Computational Neuroscience

The German Federal Ministry of Education and Research (BMBF) has established the "National Network for Computational Neuroscience" with four high-performing "Bernstein Centers for Computational Neuroscience" as the major structural elements.

The "Bernstein Award" is equipped with up to 1.25 Mio Euros in the form of a grant over a period of five years. It will be awarded to a highly qualified young researcher, considering the candidates' verifiable research profile in the field of Computational Neuroscience and the scientific concept for a future young research group. Young researchers can apply for their own position and group. The group funded by the "Bernstein Award" will become an integral part of the National Network for Computational Neuroscience. Future announcements of the "Bernstein-Award" are in the scope of the Ministry's planning.

The grant is provided for a scientific project of a young research group headed by a postdoc regardless of nationality. The project will be conducted at a German university or research institution – within or outside the Bernstein Centers. It is a prerequisite for funding that the university or research institution concerned employs the young researcher during the funding period and supports him/her with the basic equipment in terms of laboratory space and other infrastructure. A statement made to that effect by the receiving institution must be included with the project outline to be submitted.

Deadline for applications is June 2nd, 2008.

For more detailed information about the "Bernstein-Award" including application conditions please visit

www.bernstein-centers.de/en

W127920A

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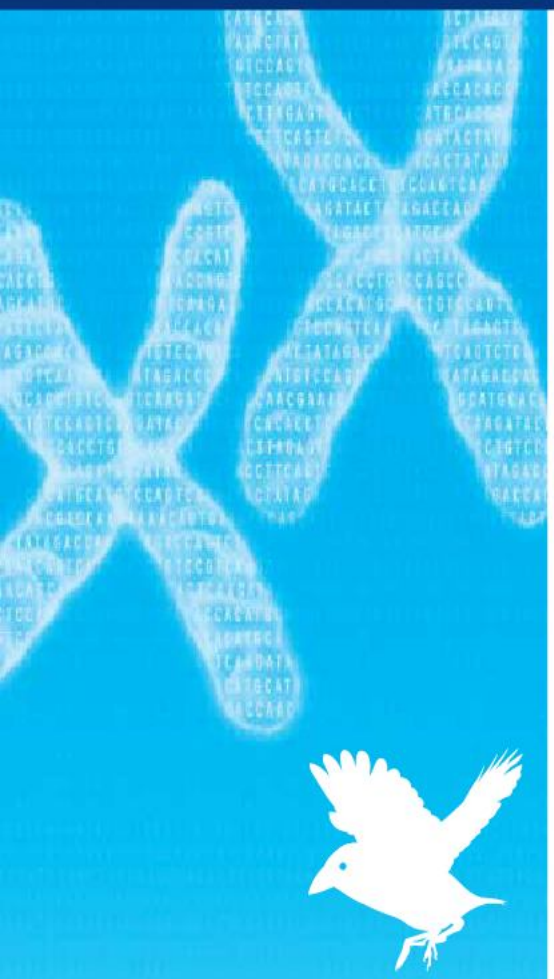
XX INTERNATIONAL CONGRESS OF GENETICS, BERLIN, 2008

BERLIN, GERMANY, JULY 12 – 17, 2008



GENETICS – UNDERSTANDING LIVING SYSTEMS

Deadline for Standard Registration Fee May 31, 2008

Genetics reveals the structure, function and evolution of living systems.

Genomics revolutionized genetic research. Now, complete annotated genome sequences are available for the human, our closest relative, the chimpanzee, and for many other model organisms. Multiple genomes have been compared and scrutinized for past and ongoing processes of variation, adaptation and speciation. Traces of the foregoing RNA world show it to be far more influential than previously suspected. Comprehensive maps of genome variation and polymorphism paint a rich picture of our population and evolutionary history and illustrate new strategies that will explain genetic, epigenetic and environmental contributions to disease risk. Transcriptomes comprehensively documenting gene expression and proteomic data sets are being built into functional networks and systems. Bioinformatics and modeling of genomic data attempt to predict and explain the functional architecture of genomes across the diversity of organisms.

The Congress in Berlin will present the latest genetic and genomic insights in ten plenary lectures and 54 concurrent symposia. 280 of the world's most prominent geneticists will speak.

For more information on the scientific program and associated activities, please visit:
http://www.geneticsberlin2008.com

Scientific Topics:
(selection)

- Aging and longevity
- Biodiversity
- Clocks and rhythms
- Computational genetics and systems biology
- Development of multicellular organisms
- Epigenetics and chromatin
- Evolutionary genomics, adaptation, speciation
- Human evolution
- Human genetics and human disease
- Metagenomics
- Neurogenetics
- RNA world
- Stem cells
- Synthetic biology

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International Genetics Federation (IGF)

BERLIN 2008 XX INTERNATIONAL CONGRESS OF GENETICS

German Genetics Society (GfG)

In partnership with nature genetics

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Fondation IPSEN, *Nature Medicine* and *Nature Immunology* present:

An Emergence & Convergence mini-symposium
Multiple Sclerosis: From Pathogenesis to Therapy

Multiple sclerosis is an inflammatory autoimmune disease targeting the central nervous system, leading to demyelination and axon degeneration and to severe disability as the disease progresses. Multiple sclerosis presents as a clinically heterogeneous disease, which has been problematic for efforts to develop appropriate animal models. Many environmental and genetic factors have been identified that may initiate disease. Various immune and neural cells have been found to play key roles in disease pathogenesis and progression. This Emergence & Convergence mini-symposium will address open questions in multiple sclerosis research, with the goal of identifying future directions that may lead to therapy.

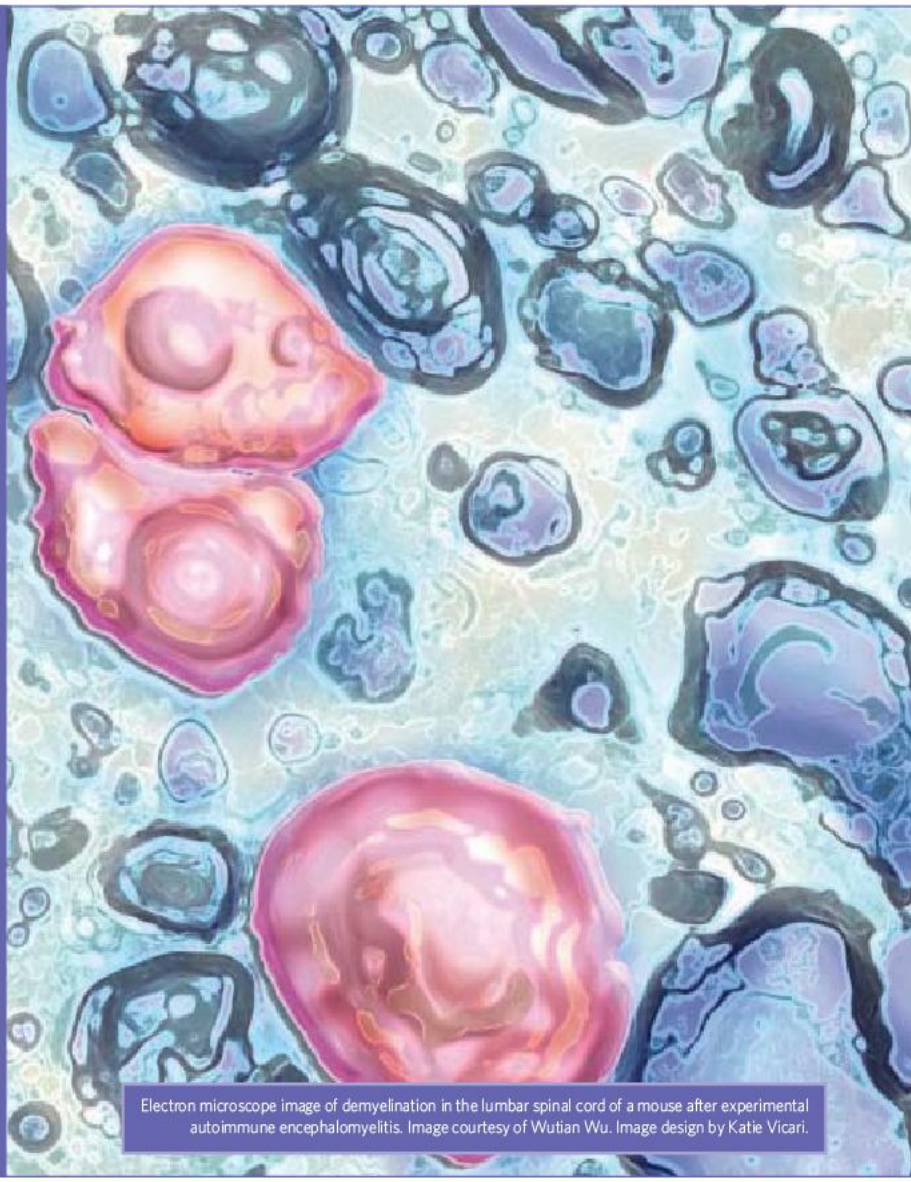
June 6, 2008
Espace Charles-Louis-Havas,
Paris, France

CHAIR
Jean-François Bach
(Hôpital Necker, France)

SPEAKERS
Burkhard Becher
(Univeristy of Zurich, Switzerland)
Christian Confavreux
(Hôpital Neurologique Pierre Wertheimer, France)
Britta Engelhardt
(University of Bern, Switzerland)
Vijay Kuchroo
(Harvard Medical School, USA)
Roland Martin
(Center for Molecular Neurobiology - Hamburg, Germany)
Stephen Sawcer
(University of Cambridge, UK)
Kenneth J. Smith
(University College London, UK)
Larry Steinman
(Stanford University, USA)

ORGANIZERS:
Eva Chmielnicki
(*Nature Medicine*, USA)
Laurie Dempsey
(*Nature Immunology*, USA)
Yves Christen
(Fondation IPSEN, France)

Application and Abstract Submission deadline:
March 31, 2008



Electron microscope image of demyelination in the lumbar spinal cord of a mouse after experimental autoimmune encephalomyelitis. Image courtesy of Wutian Wu. Image design by Katie Vicari.

Attendance at this meeting is free on acceptance of application.
To apply and for more information visit **www.nature.com/natureconferences/eandc/MS**

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PRINTED BY: St. Ives Plymouth Ltd. UK; **Publishers Press**, Lebanon Junction, Ky, USA and **Obun Printing Co. Inc**, Tokyo, Japan

Shambles

A new career for the home help.

Alexander Hay

First Lieutenant Simon Perry sighed, and leaned back in his seat. It was going to be a long day. To take his mind off things, he remembered back to a time when shambles were grown for uses other than war. How long ago it seemed.

His earliest memory was as a six-year-old, hiding behind his mother as she showed him the shamble they'd bought. The hulking, misshapen thing looked like a huge pink, melting snowman. But Perry was more scared by the look in its eyes — still vaguely human, still just-about-there.

"Say hello to your new friend, love," Mum beamed proudly. Every home should have one.

Perry grew to like his shamble. So did Arthur the dog, who barked and wagged his tail whenever the creature came indoors after helping Dad in the garden. They'd often go out to play on the green together until the rough kids from the estate came along with their pit-bulls and made trouble. Arthur was no match for any of them, human or dog. But the shamble once picked up a staffie and snapped it in two, throwing it at the feet of the owner, who had only a minute earlier been smirking as he was threatening to set Bronson on them. The gangs left Perry and his two best friends alone after that.

The backlash began a few months later. Not because a shamble could kill a man with its bare hands, but because they were driving wages down. The Trans-Human Fertilisation and Embryology Authority had ruled that medium-sized firms could have up to five shambles, and large companies could have up to ten per branch. But this, it was claimed, meant many low-skilled workers were losing their jobs. Why pay a man £10 an hour for a seven-day shift to clean loos, when you can own a shamble who'll do it for 20 hours and needs feeding only twice a day? What else needed to be said?

"And if you don't do well at school, you'll end up like them," sneered Dad when he downloaded the news one morning and pointed to the lines of charity cases left redundant. Perry nodded. He was going to be a Premier-League footballer anyway.

The shamble then lurched in from the kitchen with breakfast. Dad smiled and got it to bend down to pat it on the head. "Good boy!" he cooed, though strictly speaking it wasn't male or female. And for a while, all was well.

But then Perry's mind dwelt upon one memory in particular: the day their shamble died. They only realized there was a problem when the shamble collapsed one Sunday after dinner. Mum called the vet in, who said it was due to cellular exhaustion: the poor thing simply wasn't built to live for long. The vet finished the shamble off with an injection, and left the family alone with it for a moment. Dad was crying for only the second time in Perry's life. The first time was when poor Arthur got hit by a car and they had to put him down, too. Perry was choking it back though — Dad needed him to be strong.

JACEY

Mum just shook her head. "We can always get another one." She was always so sensible.

"Oh just bugger off!" Dad half roared, half sobbed. And he continued to stroke the shamble, tears streaming out of his eyes.

They didn't get another one in the end — it wouldn't have felt right. Instead his Dad bought a hutch and started breeding rabbits. "Keeps him out of my hair," Mum explained, while chopping carrots.

The years passed, and Perry started his A-Levels. Then the wars began. A brief nuclear exchange between India and China had killed millions, and the ensuing conventional war killed many more. The European Union was refusing to get involved, but tensions between the European capitals and India's Russian ally were growing. Perry applied for Sandhurst, and they offered him a place.

Mum was upset that he was going to be so downmarket as to become a squaddie, but Dad got excited and rang up Granddad. That afternoon, Granddad drove them up to the war memorial in town and pointed out all the old friends of his who'd died in Iraq and Afghanistan.

"Never forget, soldier, never forget …" Granddad muttered, then shook Perry by the hand. They went to the pub after that and Granddad told them about his new shamble. "Even does the shopping for me!" he said, in between sips of beer.

Russian forces poured over the EU border a year later. The casualties were high and so yet another use was found for the shambles. They were strong; they could be trained; they could have race memories encoded in their DNA; their bodies could be 'weaponized' with implants, and, best of all, if one died, another two could be grown in a month. All they needed was to be led into battle by someone they could trust, someone who sympathized with them, someone, perhaps, who'd had one as a child …

… First Lieutenant Simon Perry finished putting on his armour, his surgically altered reflexes twitching as the sounds of battle neared. Looking up, he gazed at his platoon in the APC: eighteen combat-shambles, and one trans-human sergeant, all readying their weapons for the assault. The APC rumbled to a halt and the hydraulics hissed as the exit ports opened.

Perry was the first out, looking around the ruined city as the battle screamed around them. He then gestured with his hand. Behind him, the shambles roared as one and stormed past into battle.

"Good luck, boys," he whispered as he followed them in. ■

When not slaving over a PhD thesis about online journalism, Alexander Hay's long-term goals are to become a writer and to avoid getting a proper job at all costs.

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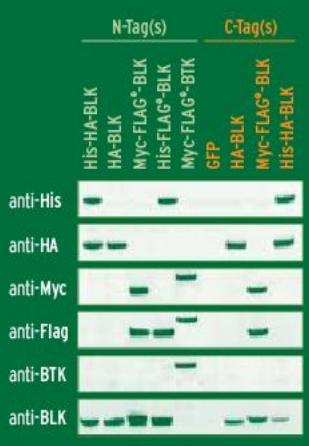

The Western blot analysis of HEK293 cell lysate over-expressing BLK or BTK tagged with indicated epitopes.

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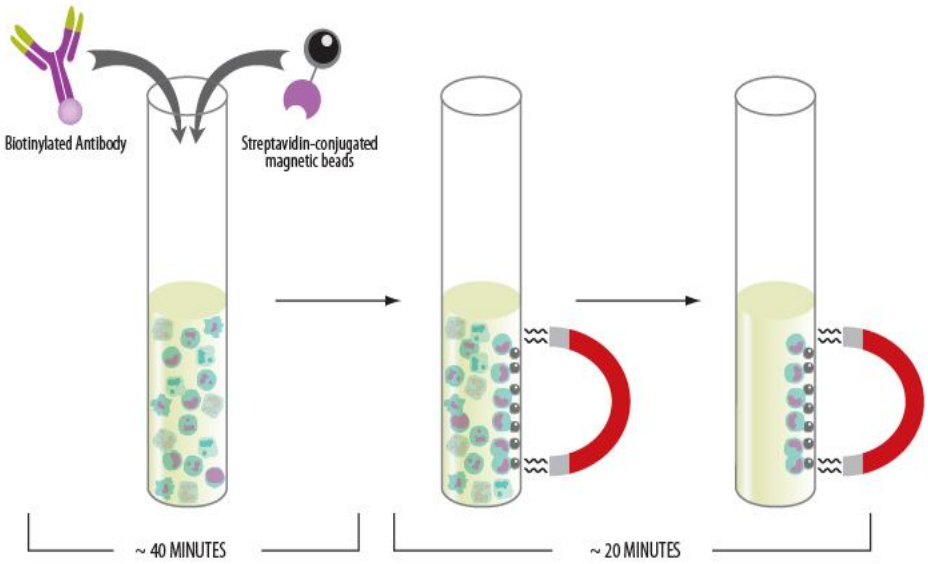

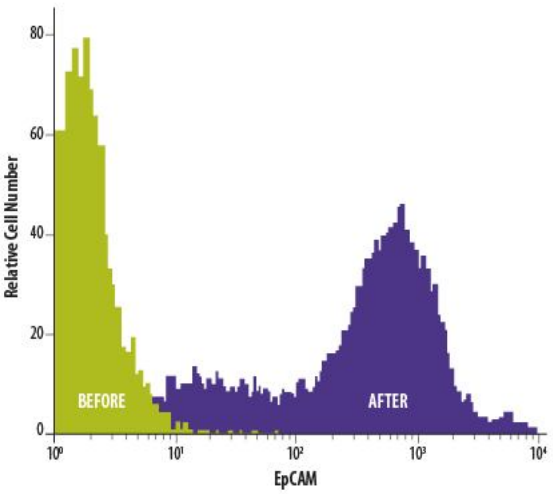

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